BMC Gastroenterology

RESEARCH

Open Access

Lipopolysaccharide-binding protein (LBP): a prognostic biomarker for gastric cancer linked to immune infiltration

Hao Lv[1,2], Mei Lv[2], Xuyang Guo[1], Xiaoman Zhu[1], Yue Chao[1] and Dandan Li[1*]

Abstract

Background Gastric cancer, characterized by rising global incidence and mortality, faces significant challenges due to the lack of effective early detection methods, delaying timely interventions and underscoring the need for novel biomarkers. Lipopolysaccharide-binding protein (LBP), implicated in cancers such as lung, colon, and cervical cancer, has emerged as a promising candidate. However, its specific roles and mechanisms in gastric cancer remain unclear, necessitating further investigation.

Methods This study utilized data from The Cancer Genome Atlas (TCGA), the Gene Expression Omnibus (GEO), and the Human Protein Atlas (HPA) to assess LBP mRNA and protein expression levels in gastric cancer patients and explore their associations with clinical outcomes. Analytical techniques included volcano plots, protein-protein interaction networks, Gene Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, gene set enrichment analysis (GSEA), and immune infiltration assessments. Furthermore, lentiviral vectors containing interference sequences targeting LBP were used to manipulate its expression in AGS and HGC-27 gastric cancer cell lines, enabling the analysis of gene knockdown effects on malignant behaviors. Western blotting (WB) was performed to validate the impact of LBP knockdown on the expression of key signaling pathway proteins.

Results Our pan-cancer comparative analysis across 33 cancer types revealed significant upregulation of LBP in gastric cancer, with diagnostic ROC curve analysis yielding an AUC of 0.765. Univariate and multivariate Cox regression analyses revealed that high LBP expression was inversely related to patient survival. Additionally, immune infiltration and functional enrichment analyses revealed the involvement of LBP in pathways crucial to cancer development, such as immune response modulation and lipid metabolism. LBP knockdown in gastric cancer cell lines reduced proliferation, migration, and invasion. WB confirmed decreased expression of P65, P-P65, STAT3, and P-STAT3 upon LBP knockdown.

Conclusion LBP is intricately linked to gastric cancer pathogenesis; it influences cell proliferation, migration, and invasion, thereby representing a valuable prognostic and diagnostic biomarker. This study not only highlights the potential of LBP as a therapeutic target but also provides the groundwork for future investigations into its mechanistic pathways in gastric cancer.

*Correspondence:
Dandan Li
lidd@jlu.edu.cn

Full list of author information is available at the end of the article

Clinical trial number Not applicable. I would like to clarify that our research does not fall under clinical studies and therefore does not involve ethical concerns related to human or animal subjects. The cells used in this study are established cell lines purchased from a certified biotechnology company. All experimental procedures comply with standard research protocols and guidelines for cell line studies.

Keywords Gastric cancer, Lipopolysaccharide-binding protein, Bioinformatics analysis, Biomarker

Introduction

Globally, gastric cancer is not only one of the deadliest malignancies but also a significant threat to human health [1]. Each year, gastric cancer results in numerous deaths, particularly in developing countries. According to statistics, the United States is expected to report 26,890 new cases of gastric cancer, with the disease ranking 15th in terms of cancer-related deaths, thereby causing an estimated 10,880 fatalities [2]. The insidious nature of gastric cancer means that it is often diagnosed at an advanced stage, at which time treatment efficacy is significantly reduced. Therefore, the early screening and diagnosis of gastric cancer are crucial [3].

In the area of stomach cancer research, lipopolysaccharide-binding protein (LBP), which is an acute-phase protein produced in the liver, has elicited considerable interest among scientists. LBP plays an essential role in the body's innate immune response, whereby it protects against infections by recognizing and binding bacterial lipopolysaccharides to activate immune cells [4–6]. Notably, recent studies have revealed connections between LBP and the development of various diseases, including cardiovascular diseases, infections, and different types of cancer, thus emphasizing the potential importance of LBP in disease biomarker research [7, 8].

For example, LBP has been implicated in several other cancers in addition to gastric cancer. Almeida et al. reported elevated levels of LBP in cancer patients with sepsis, whereby an association of LBP with systemic inflammatory responses was demonstrated in these patients [9]. In colorectal cancer, elevated plasma levels of LBP are correlated with an increased risk of this type of cancer, thereby suggesting a role for LBP in monitoring inflammatory and metabolic endotoxaemia processes linked to carcinogenesis [10, 11]. Similarly, a study by Citronberg et al. revealed that elevated plasma LBP levels are associated with increased colorectal cancer risk [12]. Furthermore, the association between LBP and cancer progression has been investigated in cancers such as ovarian cancer and lung cancer, wherein LBP was identified as being a potential biomarker for cancer diagnosis and prognosis [13–15].

In the context of stomach cancer, the role of LBP has attracted increasing interest. Castaño-Rodríguez et al. reported that polymorphisms in the LBP gene are closely associated with *Helicobacter pylori* infection and related gastric cancer risk, thus suggesting the potential of LBP as a biomarker of gastric cancer development and immune responses [16]. Additionally, Chen et al. proposed that LBP interacts with the NF-κB signalling pathway to promote immune cell infiltration and regulate the immune microenvironment of gastric cancer, thus suggesting a novel perspective on the role of LBP in immune evasion [17]. Moreover, He et al. identified LBP as a potential prognostic marker via immune-related gene differential analysis, thereby suggesting that changes in LBP may impact patient prognosis [18]. In another study, Xie et al. demonstrated that LBP derived from gastric cancer promotes liver metastasis by enhancing the formation of the premetastatic niche, thus further highlighting its critical role in gastric cancer metastasis [19].

Although previous studies have demonstrated the potential role of LBP in gastric cancer, most research has primarily focused on its relationship with immune evasion, immune responses, and metastasis. Moreover, research investigating how LBP regulates the mechanisms of gastric cancer cell proliferation, invasion, and metastasis is lacking. Although these studies have provide important insights into the association between LBP and gastric cancer, insufficient research has systematically explored the causal relationship between LBP and gastric cancer.

This study is the first to systematically evaluate LBP as a potential prognostic biomarker for gastric cancer. Via multidimensional bioinformatics analysis and the integration of clinical data, this study provides an in-depth exploration of the role of LBP in regulating gastric cancer cell proliferation, invasion, and metastasis, as well as its tumour microenvironment. The aim of this study was not only to enhance the early detection and diagnostic accuracy for gastric cancer but also to provide more precise and personalized treatment plans for patients. Furthermore, as a potential biomarker, the role of LBP in monitoring treatment responses and prognostic assessments warrants further exploration. In summary, this study seeks to offer new strategies and methods for the clinical management of gastric cancer, thereby improving patient survival rates and quality of life while providing new insights and methodologies for cancer research.

Materials and methods

Expression data acquisition and processing

In our study, we analysed RNA sequencing data from 33 different tumour types using The Cancer Genome Atlas

(TCGA) and Gene Expression Omnibus (GEO) databases, specifically by using the GSE51575 dataset. The GSE51575 dataset utilizes array technology for expression profiling analysis. The data were processed via the STAR pipeline, converted to the Transcripts Per Million (TPM) format, and underwent logarithmic normalization to enhance the robustness of the analysis [20]. Before analysis, we performed quality control on the raw data using tools such as FastQC to identify and remove low-quality samples and outliers, thus ensuring that only high-quality data were used for the subsequent analysis. We carefully managed data integrity by excluding probes that were mapped to multiple genes and selecting probes with the highest signal for singular genes. All of the procedures were performed in R software (version 4.2.1), thereby ensuring that our research met high scientific standards and providing a strong foundation for future studies [21].

Differential expression analysis of genes and diagnostic Roc curve drawing

A detailed differential expression analysis was performed on the RNA sequencing data to identify variations in the expression of the LBP gene across different samples. Given that the data followed a nonnormal distribution, the Wilcoxon signed-rank test was employed for analysis. Differential expression analysis was conducted using the stats package in R, which provides statistical methods for nonnormally distributed data. To further validate the robustness and accuracy of the analysis, the car package was also applied for regression analysis and variance analysis.

The analysis focused on pan-cancer datasets and specific gastric cancer datasets (particularly the GSE51575 dataset), with box plots being used to illustrate the differences in the expression of the LBP gene. During this process, we first standardized all of the samples and further removed extreme values and outliers using the Z score method to ensure the accuracy of the differential analysis. Additionally, the diagnostic potential of LBP in distinguishing gastric cancer samples from noncancer samples was assessed via receiver operating characteristic (ROC) analysis. Advanced visualizations, which were created using the ggplot2 package, enhanced the presentation and understanding of the results, thereby highlighting the clinical diagnostic relevance of the LBP gene.

Relationship between LBP expression and the prognosis of patients with gastric cancer

We analysed the relationship between LBP expression and gastric cancer patient prognosis by merging LBP expression data and clinical data using the Stats package in R, followed by survival analysis with the survival package. We conducted univariate and multivariate Cox regression analyses to identify and validate the factors significantly affecting prognosis. We visualized the results with Kaplan-Meier curves via the survminer and ggplot2 packages. Additionally, we assessed the prognostic efficacy of LBP via time-dependent ROC curve analysis with the timeROC package and validated our findings with a nomogram model and calibration analysis using the rms package, based on data from a previously published article in the Cell journal [22].

Single gene difference analysis and single gene correlation analysis

Afterwards, we categorized samples into high- and low-expression groups based on the expression levels of the LBP gene. To conduct differential expression analysis, we utilized the raw count matrix from a public dataset and processed the data using the DESeq2 package, according to standard procedures [23]. This step aimed to identify genes that were significantly altered due to different levels of LBP expression.

After performing the differential expression analysis, we visualized the results using the ggplot2 package. Specifically, we generated volcano plots for single-gene differential analysis to intuitively display gene expression changes and statistical significance. In these volcano plots, we set $\log FC > 1$ and $p < 0.05$ thresholds to filter out genes demonstrating significant differential expression.

We subsequently imported these differentially expressed genes into the STRING database to construct a protein-protein interaction (PPI) network [24]. By importing the PPI network results into Cytoscape software and utilizing the MCODE plugin, we identified hub genes among the differentially expressed genes. Based on these hub genes, we generated coexpression heatmaps related to LBP to further explore the potential mechanisms of LBP in gastric cancer.

To further predict the function of LBP, we conducted a correlation analysis between LBP and other molecules. We performed a Pearson correlation analysis and corrected the p values via the Benjamini-Hochberg method. Finally, we selected genes exhibiting the strongest correlation with LBP and generated coexpression heatmaps using these genes.

Functional enrichment analysis

In our functional enrichment analysis of the human (*Homo sapiens*) genome, we initially utilized the org.Hs.eg.db package for the ID conversion of the differentially expressed genes derived from single-gene differential analyses, thereby ensuring compatibility with the downstream analytical tools. Subsequent enrichment analyses via the clusterProfiler package included Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, with an aim of elucidating

the roles of differentially expressed genes in biological processes, cellular components, and molecular functions, as well as their participation in metabolic and signalling pathways. The enrichment significance was quantified via the Z score values calculated from the Log|FC| values with the GOplot package.

Additionally, gene set enrichment analysis (GSEA) was performed using clusterProfiler to assess the statistical significance of the predefined gene sets (c2.cp.all.v2022.1.Hs.symbols.gmt from the msigdbr package) within our gene list, with a focus on the elucidation of the roles of the differential genes in gastric cancer progression and their relevance in disease-associated biological pathways.

Development of cell culture and stably transfected cell lines

The human gastric cancer cell lines AGS and HGC-27 were obtained from Shanghai iCell Bioscience, Inc. The AGS cell line is derived from poorly differentiated gastric adenocarcinoma, and it is widely used in gastric cancer research because of its ability to mimic aspects of gastric tumour biology, particularly with respect to the epithelial characteristics of gastric cancer. In contrast, the HGC-27 cell line originates from a moderately differentiated gastric adenocarcinoma and is often used to examine more differentiated forms of gastric cancer. These two cell lines represent different subtypes of gastric cancer, with AGS cells being more aggressive and poorly differentiated, whereas HGC-27 cells are relatively less aggressive and more differentiated.

AGS cells were cultured in RPMI-1640 medium supplemented with 10% foetal bovine serum (FBS, Catalogue No. FS301-02, TransGen), 1% nonessential amino acids (NEAA, Catalogue No. iCell-01000, iCell), and 1% penicillin-streptomycin (Catalogue No. P1400, Solarbio). HGC-27 cells were maintained in RPMI-1640 medium enriched with 10% FBS and 1% penicillin-streptomycin. Both cell types were cultured in a humidified atmosphere at 37 °C with 5% CO_2.

To construct the pLKO.1-Scramble and pLKO.1-shLBP plasmids, we utilized the lentiviral vector plasmid pLKO.1-Puro (Catalogue No. FH1717, Shanghai Fenghui Biotechnology Co., Ltd.). The utilized interference sequences were shLBP 5'-AGUUUCAGGAAGGAUUUGCGC-3' and scramble 5'-GTATAAGTCAACTGTTGAC-3'. The utilized lentiviruses in this study were produced using a three-plasmid packaging system. The lentiviral vector plasmid was cotransfected with the packaging plasmid PMD2.G (Catalogue No. BR037, Fenghui) and psPAX2 (Catalogue No. BR036, Fenghui), along with the Lipofectamine™ 3000 transfection reagent (Catalogue No. L3000150, Thermo Fisher Scientific), into HEK-293T cells (Catalogue No. iCell-h237, iCell). The medium was collected at 48 and 72 h posttransfection, filtered through a 0.22 μm filter, and stored at 4 °C for up to one week.

For the generation of stably transfected cell lines, AGS and HGC-27 cells were seeded in 6-well plates (300,000 cells per well). After 24 h, 1 mL of the abovementioned prepared lentivirus-containing medium was added to each well. The medium was replaced after 48 h. Subsequently, cells harbouring the lentivirus-encoded puromycin resistance gene were selected with 2 μg/mL puromycin. This selection with puromycin continued for one week prior to cell collection and further analyses, thereby ensuring the establishment of stably transfected cell lines.

Real-time PCR

Total RNA was extracted from stably transfected cells using the EasyPure RNA Kit (Catalogue No. ER101-01, TransGen), and first-strand cDNA was synthesized using the cDNA Synthesis Kit (Catalogue No. AT311-02, TransGen) according to the manufacturer's instructions. Real-time quantitative PCR (qPCR) was conducted using the SYBR Green qPCR Kit (Catalogue No. AQ132, TransGen) following the manufacturer's protocol, with GAPDH serving as the internal reference gene. The utilized primers were as follows.

For the LBP gene, the forward primer was 5'-TGGCTGTTGAACCTCTTCCA-3', and the reverse primer was 5'-TGTCGGCGAAACTGTCAATC-3'. For the GAPDH gene, the forward primer was 5'-GAAGGTGAAGGTCGGAGTC-3', and the reverse primer was 5'-GAAGATGGTGATGGGATTTC-3'.

PCR amplification was performed on the ABI-Q3 system. The reaction was initiated with a 30-second denaturation at 94 °C, followed by 45 cycles of 5 s at 94 °C, 15 s at 51 °C, and 10 s at 72 °C (Thermo Fisher Scientific, Inc.). The mRNA expression levels were quantified using the 2-ΔΔCq method.

Cell counting Kit-8 (CCK-8) assay

AGS, HGC-27, AGS-shScramble, HGC-27-shScramble, AGS-shLBP, and HGC-27-shLBP cells were seeded in 96-well plates at a density of 3,000 cells per well. At 24, 48, and 96 h postcultivation, 10 μL of CCK-8 solution (Catalogue No. BA00208, Bioss) was added to each well. After incubation at 37 °C for 1.5 h, the absorbance of each well at 450 nm was measured using a microplate reader (Detie, Inc., model E0226).

Invasion assay

To assess the invasive potential of the aforementioned gastric cancer cells, we utilized Transwell chambers (Catalogue No. 14342, Labselect) with surfaces coated with Matrigel (Catalogue No. 356234, BD Biosciences) following the manufacturer's guidelines. A total of 3 × 10^4

cells were suspended in 100 μL of serum-free RPMI-1640 medium and seeded into the upper chamber, whereas the lower chamber was filled with 600 μL of medium containing 10% foetal bovine serum. After incubation at 37 °C for 30 h, residual cells on the upper surface were removed with a cotton swab, and invasive cells were stained with 10% Giemsa stain. Images were captured using an optical microscope (AE2000, Motic).

Wound-healing assay

To evaluate the migratory capabilities of the aforementioned gastric cancer cells, we conducted wound healing assays. Cells seeded in six-well plates (3 × 10^5 cells per well) were subjected to linear scratches using the tip of a 200 μL pipette. The cells were subsequently washed with PBS to remove detached cells. Photographs were taken at 24 and 48 h postscratch by using a digital camera and an optical microscope (Motic) to observe cell migration into the scratched area. All of the microscopy images were captured at consistent magnifications and time points.

Colony formation assay

AGS and HGC-27 cells were seeded in six-well plates at a density of 100 cells per well. After 10 days, typical colony formation was observed. The cells were fixed with methanol and then stained with 10% Giemsa (Biotopped, China). The number of visible colonies was counted to assess the clonogenic ability of the cells. All of the experiments were conducted in triplicate to ensure reproducibility.

Western blotting analysis

Total cellular proteins were extracted from collected cell samples using RIPA lysis buffer (Servicebio, China) supplemented with PMSF. After centrifugation, the supernatant was collected, and protein concentrations were determined using a BCA protein assay kit (Servicebio, China). Equal amounts of protein were mixed with loading buffer (Servicebio, China) and separated by SDS-PAGE gel electrophoresis at 80 V for 10 min, followed by 150 V for 60 min. The proteins were then transferred to PVDF membranes at 300 mA for 45 min using a transfer apparatus.

The membranes were incubated overnight at 4 °C with the following primary antibodies: rabbit anti-GAPDH (1:10,000, GB15004, Servicebio), rabbit anti-LBP (1:1,000, GB113205, Servicebio), rabbit anti-P65 (1:1,000, GB11997, Servicebio), rabbit anti-P-P65 (1:1,000, GB113882, Servicebio), rabbit anti-STAT3 (1:1,000, GB11176, Servicebio), and rabbit anti-P-STAT3 (1:1,000, GB150001, Servicebio). After incubation, the membranes were washed three times with TBST for 5 min each and then incubated with HRP-conjugated goat anti-rabbit secondary antibody (1:3,000, GB23303, Servicebio) for 1 h at room temperature. Following secondary antibody incubation, the membranes were washed again with TBST to remove any residual secondary antibody. Finally, target protein bands were visualized using an enhanced chemiluminescence reagent kit (Servicebio, China) and normalized to the internal control.

Statistical analysis

In this study, statistical analyses are presented as the mean values and standard deviations (SDs) of three independent experiments. These analyses were conducted using SPSS software version 23.0 or R language version 3.6.3. Intergroup differences were evaluated using one-way analysis of variance (ANOVA) supplemented by the Dunnett's post hoc test, the Kruskal-Wallis test, or the Student's t test, as appropriate. Differences were considered statistically significant when the p value was less than 0.05.

Results

Pan-cancer analysis of LBP expression differences across 33 types of tumours

Figure 1A and B present the results of the pan-cancer analysis of unpaired and paired samples, respectively. This study conducted a comprehensive differential expression analysis of RNA sequencing data from 33 types of tumours in TCGA, thus exploring the expression pattern of LBP in these tumours. Unpaired sample analysis revealed that LBP was significantly upregulated in bladder cancer (BLCA), colorectal cancer (COAD), glioblastoma (GBM), lung adenocarcinoma (LUAD), squamous cell lung cancer (LUSC), rectal cancer (READ), and stomach cancer (STAD) samples compared with normal samples. Conversely, it was significantly downregulated in breast cancer (BRCA), cholangiocarcinoma (CHOL), adrenocortical carcinoma (KICH), renal pelvis cancer (KIRP), pheochromocytoma and paraganglioma (PCPG), and thyroid cancer (THCA) samples. Compared with that in normal tissues, LBP expression was significantly increased in BLCA, COAD, renal cell carcinoma (KIRC), LUAD, and STAD samples but significantly decreased in BRCA, CHOL, KICH, and KIRP samples. The sample sizes for each tumour group that was used in the pan-cancer analysis are shown in Table 1. The differential expression pattern suggests that LBP may play a significant biological role in the development and progression of the aforementioned tumours, thereby warranting further explorations of its specific functions and mechanisms within the tumour microenvironment.

Differential expression analysis of LBP in stomach cancer

By utilizing the latest RNA-seq data from TCGA for stomach cancer, differential expression analysis revealed significant overexpression of LBP in tumour samples,

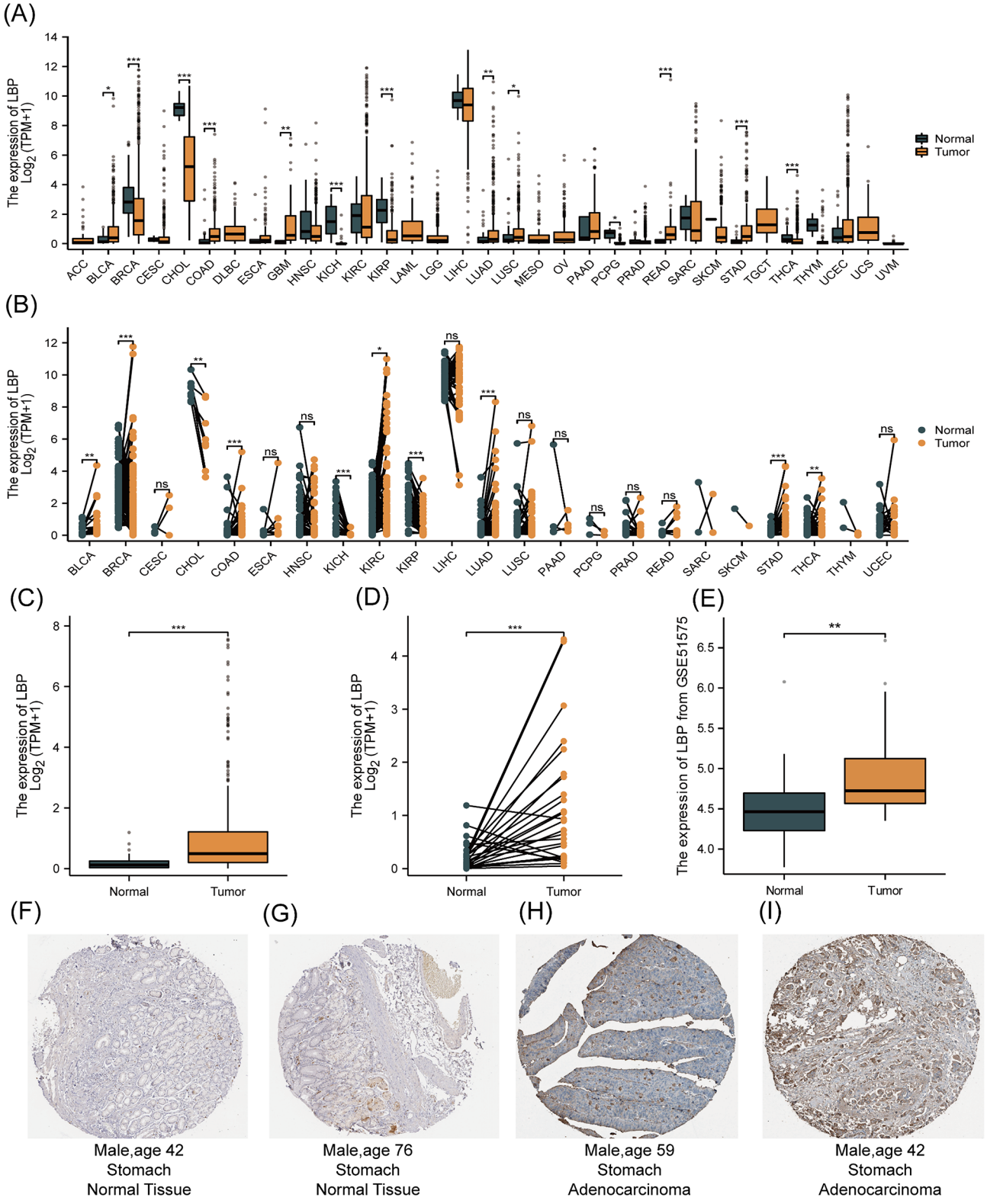

Fig. 1 Differential Expression Analysis of LBP in 33 Pan-cancer Types and Gastric Cancer. (**A**) Pan-cancer analysis of paired samples. (**B**) Pan-cancer analysis of non-paired samples. (**C**) Differential expression analysis of unpaired samples. (**D**) Differential expression analysis of paired samples. (**E**) Differential expression analysis of GSE51575 dataset. (**F**-**I**) Immunohistochemical analysis of LBP expression in normal and gastric cancer tissues

Table 1 Samples included in pan-cancer analysis of paired and unpaired samples

Category	No. Paired samples		No. Non-paired samples	
	Adjacent	Tumor	Normal	Tumor
ACC			128	
BLCA	19	19	28	407
BRCA	112	112	292	1099
CESC			13	306
CHOL	9	9	9	36
COAD	26	26	349	290
DLBC			444	47
ESCA	13	13	666	182
GBM			1157	166
HNSC	43	43	44	520
KICH	25	25	53	66
KIRC	72	72	100	531
KIRP	32	32	60	289
LAML			70	173
LGG			1152	523
LIHC	50	50		371
LUAD	58	58	347	515
LUSC	50	50	338	498
MESO				87
OV			88	427
PAAD	4	4	171	179
PCPG			3	182
PRAD	52	52	152	496
READ	6	6	318	93
SARC			2	262
SKCM			813	469
STAD	33	33		414
TGCT			165	154
THCA	59	59	338	512
THYM			446	119
UCEC	7	7	101	181
UCS			78	57
UVM				79

which was demonstrated both in the unpaired (Fig. 1C) and paired (Fig. 1D) samples, as shown in Fig. 2. To further validate this result, the GSE51575 dataset from the GEO database (comprising 26 samples each of tumour and normal tissues) was analysed, thus confirming the findings from TCGA (Fig. 1E).

Immunohistochemical validation of LBP expression in stomach cancer

Figures 1F-I show the results of the immunohistochemical analysis of LBP expression in normal and stomach cancer tissues, which utilized data from the HPA database. Consistent with the transcriptome results, LBP expression was significantly higher in tumour tissues (Fig. 1H, I) than in normal tissues (Fig. 1F, G), thus suggesting that LBP may play a crucial role in the onset and progression of stomach cancer.

Evaluation of differential expression analysis results using ROC curves

The diagnostic efficacy of LBP as a tumour biomarker was assessed via ROC curve analysis, which was used to calculate the true positive rate (sensitivity) and false positive rate (specificity) at different thresholds. The aim of this analysis was to determine the diagnostic threshold for differentiating patients with tumours from the control group. As shown in Fig. 3A, the area under the ROC curve (AUC) for LBP was 0.765, thereby indicating good diagnostic performance for distinguishing patients from controls. Generally, an AUC value greater than 0.9 indicates excellent diagnostic tests, 0.7 to 0.9 is considered to be good, 0.6 to 0.7 is considered to be poor, and values less than 0.6 are deemed to have no diagnostic value. The AUC value of 0.765 significantly exceeded the random chance level of 0.5, thereby suggesting that LBP could serve as an effective biomarker for differentiating tumour patients from nontumour individuals and highlighting its potential clinical application in diagnosing specific tumours.

LBP overexpression indicates poor prognosis in gastric cancer patients

To further explore the role of LBP in the development and progression of gastric cancer and to clarify whether the high expression of LBP represents a risk factor for patients with gastric cancer, we conducted a prognostic analysis by using clinical and survival information from gastric cancer patients in TCGA. Initially, patients were divided into the following two groups based on the expression of LBP: those with expression levels below the median expression level were categorized into the low-expression group, and those with levels above the median expression level were categorized into the high-expression group. This classification involved 375 patients with baseline characteristics that are presented in Table 2. The baseline data suggested that there was no direct correlation observed between LBP expression and clinical characteristics.

To more thoroughly explore the relationship between LBP expression and the clinical characteristics of gastric cancer patients, we further employed univariate and multivariate Cox regression analyses with the aforementioned clinical data to assess the impacts of various clinicopathological features on overall survival rates. The results (shown in Table 3) indicated that in the univariate analysis, pathological T stage (T3, T2, and T4 compared with T1), N stage (N1, N2, and N3 compared with N0), M stage (M1 compared with M0), and overall pathological stage (Stages III and IV compared with Stages I and

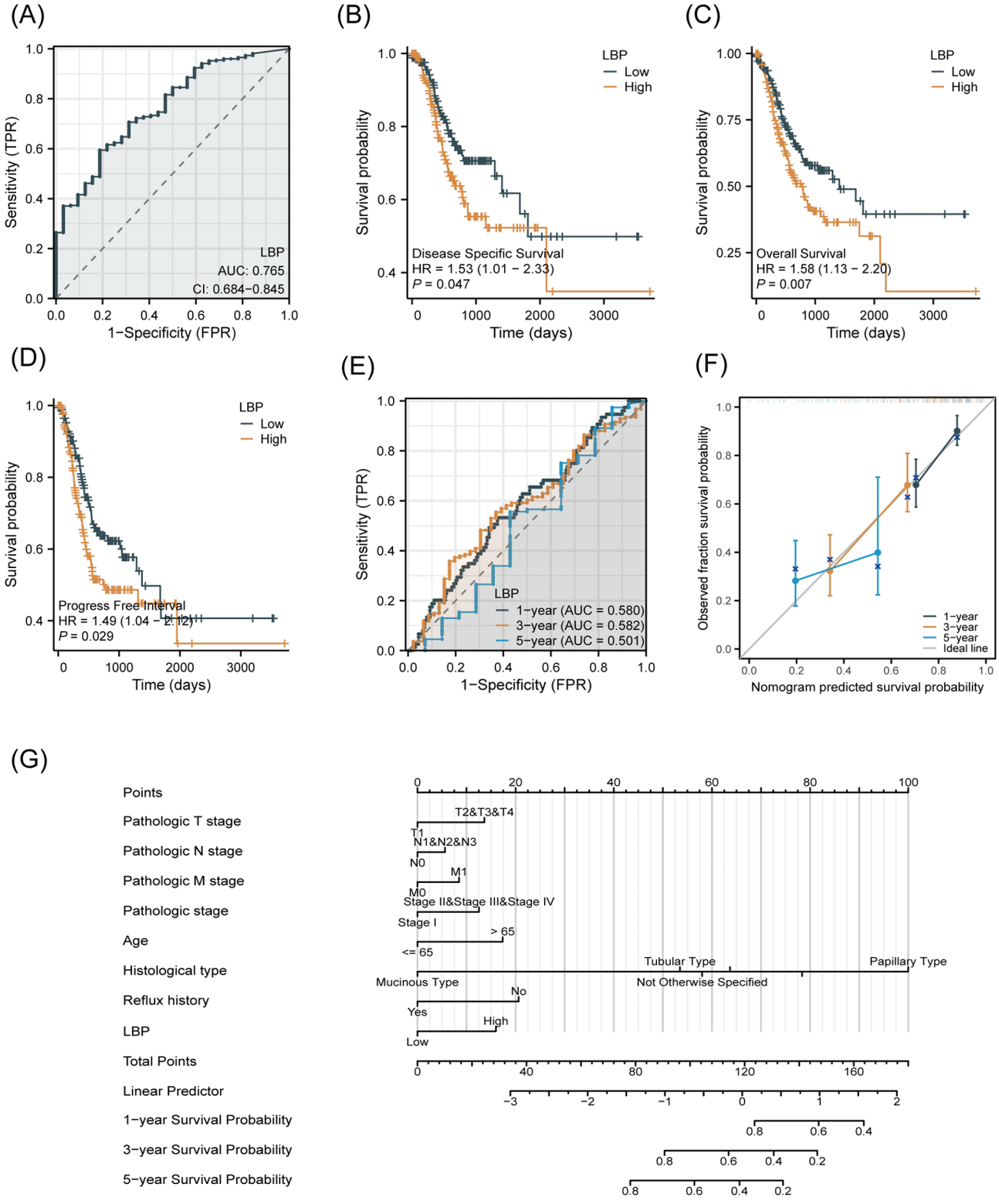

Fig. 2 Single-Gene Differential Expression Analysis, Correlation Analysis, and Construction of the PPI Network and Co-expression Heatmap. (**A**) Single-gene differential expression analysis: Volcano plot. (**B**) Single-gene differential expression analysis: Differential expression plot. (**C**) PPI network of hub genes. (**D**, **E**) Co-expression heatmap between hub genes and LBP. (**F**) Co-expression heatmap of the top 35 genes most strongly correlated with LBP

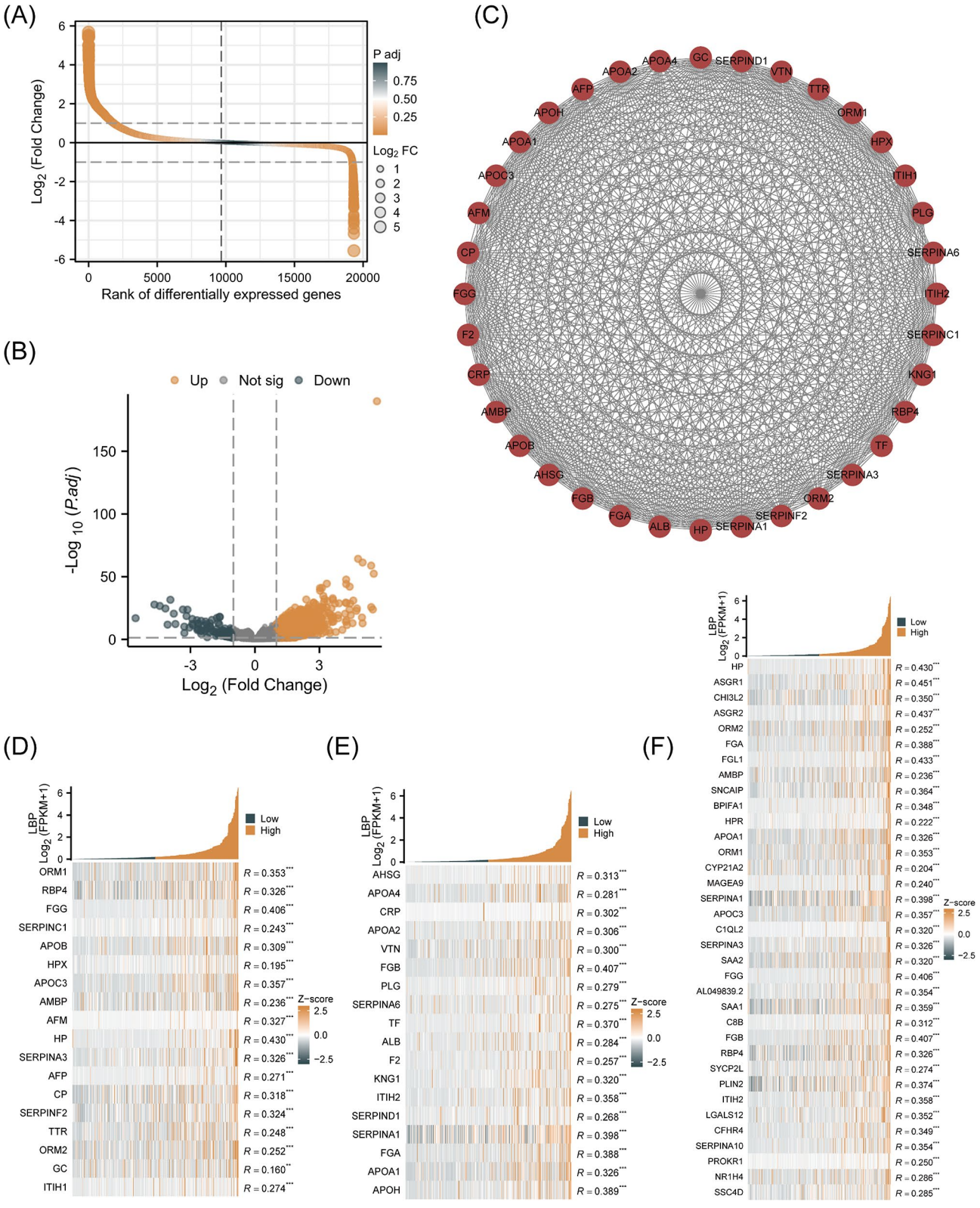

Fig. 3 Impact of High LBP Expression on Prognosis in Gastric Cancer Patients and Construction of a Nomogram for Clinical Diagnosis. (**A**) Diagnostic ROC curve. (**B**) Kaplan-Meier (KM) survival analysis results: Overall survival. (**C**) Kaplan-Meier (KM) survival analysis results: Disease-free survival. (**D**) Kaplan-Meier (KM) survival analysis results: Disease-specific survival. (**E**) Time-dependent ROC curve analysis. (**F**) Calibration analysis results. (**G**) Nomogram for clinical prognostic prediction.Single-Gene Differential Expression Analysis and Correlation Analysis

Table 2 The baseline data table including 375 gastric cancer patients

Characteristics	Low expression of LBP	High expression of LBP	*P* value
n	187	188	
Pathologic T stage, n (%)			0.268
T1&T2	53 (14.4%)	46 (12.5%)	
T3	77 (21%)	91 (24.8%)	
T4	55 (15%)	45 (12.3%)	
Pathologic N stage, n (%)			0.518
N0	60 (16.8%)	51 (14.3%)	
N1	47 (13.2%)	50 (14%)	
N2	41 (11.5%)	34 (9.5%)	
N3	33 (9.2%)	41 (11.5%)	
Pathologic M stage, n (%)			0.142
M0	169 (47.6%)	161 (45.4%)	
M1	9 (2.5%)	16 (4.5%)	
Pathologic stage, n (%)			0.486
Stage I	29 (8.2%)	24 (6.8%)	
Stage II	58 (16.5%)	53 (15.1%)	
Stage III	78 (22.2%)	72 (20.5%)	
Stage IV	15 (4.3%)	23 (6.5%)	
Gender, n (%)			0.264
Female	72 (19.2%)	62 (16.5%)	
Male	115 (30.7%)	126 (33.6%)	
Age, n (%)			0.642
<= 65	80 (21.6%)	84 (22.6%)	
> 65	106 (28.6%)	101 (27.2%)	
Histological type, n (%)			0.600
Diffuse Type	37 (9.9%)	26 (7%)	
Mucinous Type	8 (2.1%)	11 (2.9%)	
Papillary Type	3 (0.8%)	2 (0.5%)	
Signet Ring Type	4 (1.1%)	7 (1.9%)	
Tubular Type	34 (9.1%)	35 (9.4%)	
Not Otherwise Specified	100 (26.7%)	107 (28.6%)	
Histologic grade, n (%)			0.186
G1	4 (1.1%)	6 (1.6%)	
G2	61 (16.7%)	76 (20.8%)	
G3	118 (32.2%)	101 (27.6%)	
Anatomic neoplasm subdivision, n (%)			0.618
Antrum/Distal	71 (19.3%)	67 (18.3%)	
Cardia/Proximal	21 (5.7%)	27 (7.4%)	
Fundus/Body	65 (17.7%)	65 (17.7%)	
Gastroesophageal Junction	21 (5.7%)	20 (5.4%)	
Other	2 (0.5%)	2 (0.5%)	
Stomach (NOS)	5 (1.4%)	1 (0.3%)	
Reflux history, n (%)			0.203
No	88 (41.1%)	87 (40.7%)	
Yes	24 (11.2%)	15 (7%)	
Antireflux treatment, n (%)			0.934
No	68 (38%)	74 (41.3%)	
Yes	18 (10.1%)	19 (10.6%)	
H pylori infection, n (%)			0.934
No	74 (45.4%)	71 (43.6%)	
Yes	9 (5.5%)	9 (5.5%)	
Barretts esophagus, n (%)			0.267

Table 2 (continued)

Characteristics	Low expression of LBP	High expression of LBP	*P* value
No	100 (48.1%)	93 (44.7%)	
Yes	10 (4.8%)	5 (2.4%)	

[a] Data incomplete as some record data were lost

Table 3 The results of univariate and multivariate Cox regression analysis

Characteristics	Total(*N*)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Pathologic T stage	362				
T1	18	Reference		Reference	
T3&T2&T4	344	8.829 (1.234–63.151)	**0.030**	1.839 (0.235–14.367)	0.561
Pathologic N stage	352				
N0	107	Reference		Reference	
N1&N2&N3	245	1.925 (1.264–2.931)	**0.002**	0.835 (0.359–1.938)	0.674
Pathologic M stage	352				
M0	327	Reference		Reference	
M1	25	2.254 (1.295–3.924)	**0.004**	1.238 (0.457–3.352)	0.674
Pathologic stage	347				
Stage I&Stage II	160	Reference		Reference	
Stage III&Stage IV	187	1.947 (1.358–2.793)	**< 0.001**	2.143 (0.998–4.604)	0.051
Gender	370				
Female	133	Reference			
Male	237	1.267 (0.891–1.804)	0.188		
Age	367				
<= 65	163	Reference		Reference	
> 65	204	1.620 (1.154–2.276)	**0.005**	1.843 (1.123–3.026)	**0.016**
Histological type	369				
Diffuse Type	63	Reference		Reference	
Mucinous Type	19	0.288 (0.087–0.954)	**0.042**	0.092 (0.012–0.716)	**0.023**
Not Otherwise Specified	202	1.179 (0.751–1.852)	0.475	0.845 (0.436–1.636)	0.617
Papillary Type	5	1.705 (0.514–5.663)	0.383	4.017 (1.087–14.844)	**0.037**
Signet Ring Type	11	2.430 (1.093–5.404)	**0.029**	1.627 (0.562–4.704)	0.369
Tubular Type	69	0.953 (0.548–1.655)	0.863	0.641 (0.321–1.281)	0.208
Histologic grade	361				
G1	10	Reference			
G2&G3	351	1.957 (0.484–7.910)	0.346		
Anatomic neoplasm subdivision	362				
Antrum/Distal	138	Reference			
Cardia/Proximal	47	1.275 (0.786–2.069)	0.324		
Fundus/Body	129	0.957 (0.646–1.416)	0.825		
Gastroesophageal Junction	39	0.800 (0.426–1.501)	0.486		
Other&Stomach (NOS)	9	0.371 (0.090–1.528)	0.170		
Reflux history	213				
No	174	Reference		Reference	
Yes	39	0.582 (0.291–1.162)	0.125	0.464 (0.205–1.050)	0.065
H pylori infection	162				
No	144	Reference			
Yes	18	0.650 (0.279–1.513)	0.317		
LBP	370				
Low	185	Reference		Reference	
High	185	1.579 (1.134–2.199)	**0.007**	1.698 (1.062–2.716)	**0.027**

[a] Data incomplete as some record data were lost

II) had significant impacts on survival. Age (>65 years compared with ≤65 years) was also identified as being a significant factor. In the multivariate analysis, both age and LBP expression level (high versus low) retained their significance, thereby suggesting that these variables could play crucial roles in disease prognosis. Notably, high LBP expression was significantly associated with reduced patient survival rates, thus indicating that LBP can serve as an independent prognostic biomarker.

We subsequently conducted an in-depth analysis using the Kaplan-Meier (KM) survival method to further evaluate the effectiveness of LBP as a potential independent prognostic marker. The analysis (Fig. 3B, C, D) revealed that, compared with the low-expression group, the high-expression group demonstrated significantly lower survival rates and shorter survival times in terms of overall survival (OS), progression-free interval (PFI), and disease-specific survival (DSS), with p values for all three datasets being less than 0.05, thereby indicating statistically significant differences. These findings align with the results of the Cox regression analysis and further support the potential of LBP as an independent prognostic indicator.

The clinical prognostic prediction tool constructed by using LBP expression values

In this study, time-dependent ROC curve analysis was employed to assess the accuracy of LBP in predicting patient survival rates. The analysis aimed to evaluate the ability of LBP as a biomarker for predicting patient survival at various time points, with a focus on one-, three-, and five-year survival predictions.

The results (Fig. 3E) revealed that the area under the curve (AUC) for LBP was 0.580 at one year, 0.582 at three years, and 0.501 at five years. These findings indicate that, as a prognostic predictor, LBP exhibits some predictive value in the short term (one and three years) with modest accuracy (AUC values slightly greater than 0.5). However, at five years, the predictive ability of LBP approached a random level (AUC of 0.501), thus suggesting limited effectiveness of LBP in long-term prognostic prediction.

In conclusion, although LBP is somewhat associated with short-term patient survival, its potential as an independent biomarker for long-term survival prediction is limited. These insights elicit caution against the sole reliance on LBP for clinical prognostic assessments and suggest that combining this factor with other biomarkers or clinical parameters may increase prediction accuracy.

Elaborating upon these findings, this study integrated multivariate Cox regression analysis results to develop a more effective prognostic prediction model for gastric cancer patients. By considering various clinical and pathological factors in the multivariate analysis, a nomogram for the clinical prognostic prediction tool was successfully constructed. The tool's efficacy was further validated via calibration analysis (Fig. 3F), thereby confirming its applicability in the prognostic prediction of gastric cancer. The nomogram provides clinicians with an intuitive, easy-to-use method for more accurately predicting the survival prognosis of gastric cancer patients, as illustrated in Fig. 3G.

To elucidate the function of LBP in gastric cancer progression, patients were stratified into high- and low-LBP expression groups. Differential expression analysis, which utilized RNA-seq data from these cohorts, aimed to identify the pathways and functions associated with elevated LBP levels. The analysis (with thresholds of $|LogFC|>1$ and $p.adj<0.05$) revealed 1,895 differentially expressed genes, including 1,791 upregulated and 104 downregulated genes (Fig. 2A, B).

In this study, the STRING database was used to construct a protein-protein interaction (PPI) network of these genes, thereby facilitating a comprehensive understanding of their biological interactions and potential functional relationships.

Key hub genes within the PPI network were identified using the MCODE plugin, which highlighted 36 significant genes, including ORM1, RBP4, and others (detailed in Fig. 2C). A coexpression heatmap of these hub genes with LBP is depicted in Fig. 2D and E, with the aim of revealing their roles in gastric cancer and their influence on disease biology.

Furthermore, a single-gene correlation analysis identified genes with the strongest association with LBP, thereby aiding in the creation of a coexpression heatmap (Fig. 2F). This analysis identified the top 35 genes correlated with LBP, such as HP, ASGR1, and CHI3L2, thus providing insights into the specific mechanisms and pathways of LBP in gastric cancer progression. This approach is pivotal for advancing precision medicine in gastric cancer, thus suggesting potential therapeutic targets.

Immune infiltration analysis suggests that LBP is crucial for tumour immune surveillance and immune therapy response

As the prominence of immunotherapy in tumour treatments continues to increase, the exploration of the tumour immune microenvironment has emerged as a pivotal area of contemporary research. This development is especially true with respect to gastric cancer treatment, wherein the elucidation of the role of LBP in the immune infiltration process becomes crucial. Accordingly, this study focused on an analysis of immune infiltration, with the aim of delineating the immunological functions of LBP in gastric cancer.

The findings of our analysis revealed a significant correlation between the differential expression of LBP and

infiltration by specific immune cell types (Fig. 4A). This insight provides new avenues for understanding how LBP may influence tumour immune responses. Furthermore, our study revealed a notable positive correlation between LBP expression and a cohort of essential immune-modulatory molecules, including the angiogenesis regulator ANGPT2, fibrinogen-like protein 1 (FGL1), immunoglobulin superfamily member 1 (IGSF1), NLR family pyrin domain containing 12 (NLRP12), and serum amyloid A2 (SAA2) (Figs. 4B-F). These correlations

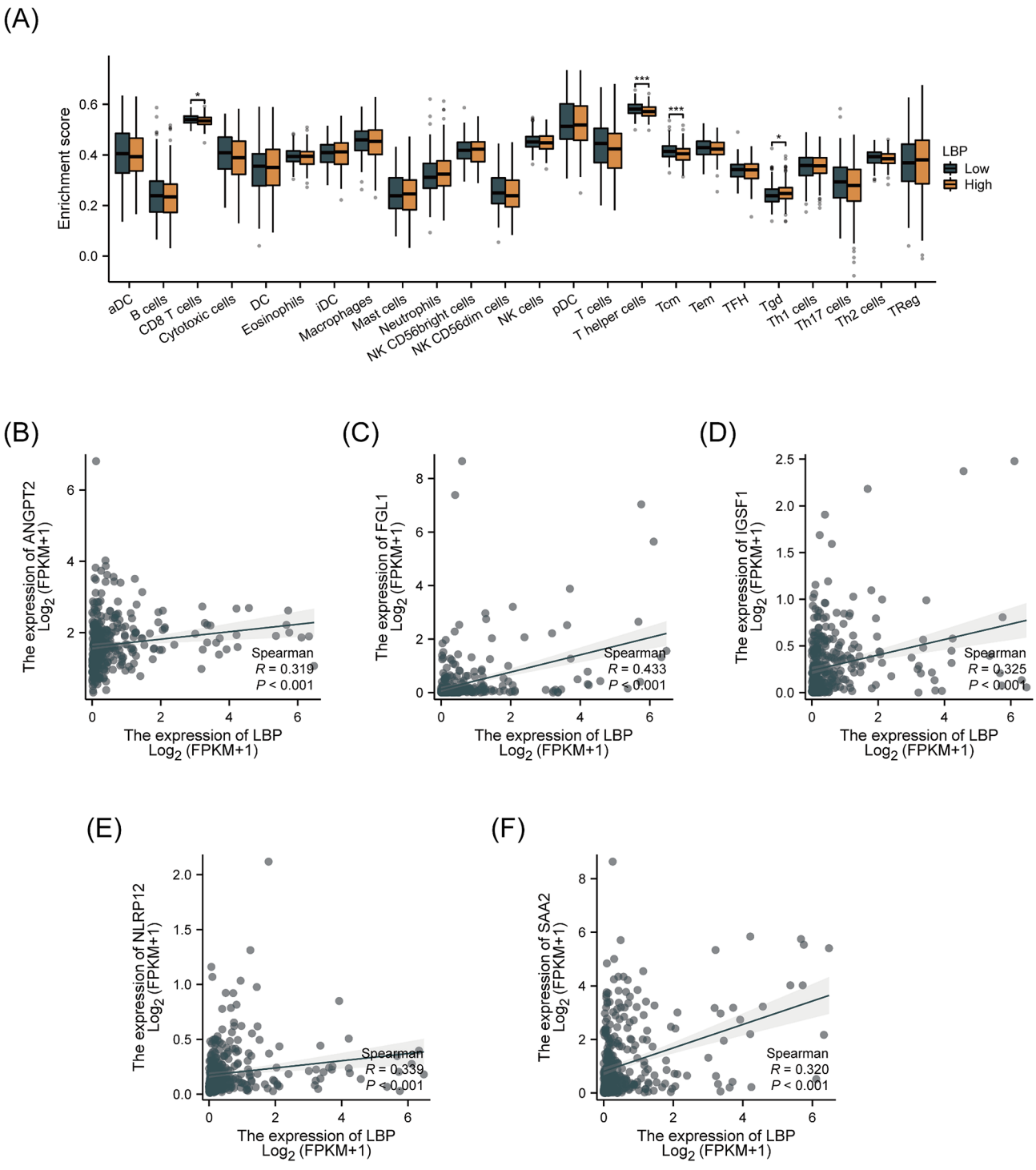

Fig. 4 Immune Infiltration Analysis and Correlation Between LBP and Common Immune Therapy Targets. (**A**) Group comparison of immune cell infiltration in patients with different LBP expression profiles. (**B**) Correlation between LBP and ANGPT2. (**C**) Correlation between LBP and FGL1. (**D**) Correlation between LBP and IGSF1. (**E**) Correlation between LBP and NLRP12. (**F**) Correlation between LBP and SAA2

suggest a significant role for LBP in the immune infiltration dynamics of gastric cancer, which is potentially achieved by modulating immune responses within the tumour microenvironment via its interaction with these critical molecules.

Functional enrichment analysis predicts the role of LBP in gastric cancer progression

To explore the potential functions of LBP in gastric cancer, this study conducted analyses based on differential gene expression. These included GO analysis (detailed in Fig. 5A; Table 4), KEGG analysis (detailed in Fig. 5B; Table 5), and GSEA (detailed in Figs. 5C-E; Table 6) to comprehensively elucidate the involvement of LBP in biological processes, cellular components, and molecular functions during gastric cancer development.

Key findings from the GO analysis for the biological process (BP): LBP was shown to be significantly associated with multiple processes, including serotonin receptor signalling, G protein-coupled receptor signalling related to cyclic nucleotide second messengers, DNA methylation in gamete generation, and the bacterial defence response.

For Cellular component (CC), LBP was shown to be associated with high-density lipoprotein particles, plasma lipoprotein particles, myofibrils, GABA receptor complexes, and ion channel complexes.

For molecular function (MF), LBP was shown to be linked to neurotransmitter receptor activity, endopeptidase inhibitor activity, and serotonin receptor activity.

KEGG analysis revealed the associations of LBP with several crucial pathways, including neuroactive ligand-receptor interactions; moreover, LBP may regulate neurotransmitter-receptor interactions.

For complement and coagulation cascades, LBP was demonstrated to play a role in complement activation and coagulation.

With respect to steroid hormone biosynthesis, LBP may influence steroid hormone synthesis.

Other pathways included pancreatic secretion, phototransduction, vitamin digestion and absorption, fat digestion and absorption, and bile secretion, thereby suggesting that LBP is involved in various digestive and metabolic processes.

These results emphasize the multifaceted roles of LBP in gastric cancer, particularly regarding neuroregulation, the immune response, lipid metabolism, and gene regulation. These findings provide valuable clues for further research on the role of LBP in gastric cancer development.

Knockdown of the expression of LBP in gastric cancer cell lines and its impact on the proliferation, invasion, and metastasis of gastric cancer cells

To elucidate the efficacy of LBP as a prospective therapeutic target in gastric cancer, this study employed shRNA-mediated knockdown to suppress LBP expression in the gastric cancer cell lines AGS and HGC-27. Observational experiments were conducted to assess the effects of LBP suppression on cell proliferation, invasion, and metastasis. The knockdown efficiency was initially confirmed via quantitative PCR (qPCR) analysis. Figure 6A shows a significant decrease in LBP expression in the shLBP group compared with both the MOCK and scramble groups, thereby confirming the effective knockdown of LBP. This study sought to determine the impact of LBP suppression on the proliferative capabilities of gastric cancer cells via CCK-8 proliferation assays and colony formation tests. As shown in Figures B, C, D and F, a marked reduction in the proliferation of cells within the shLBP group was observed, with these differences being statistically significant. Moreover, this study explored the ramifications of LBP knockdown on cellular migration via wound healing assays. As depicted in Figs. 6H, I and J, the results revealed a significant attenuation in the migratory potential of gastric cancer cells after LBP reduction. Finally, Transwell invasion assays were used to determine the influence of LBP knockdown on the invasive properties of gastric cancer cells. In alignment with the preceding results, cells exhibiting diminished LBP expression demonstrated a notably reduced invasion capability, as shown in Fig. 6E and G, thus further substantiating the potential of LBP as a viable target in the therapeutic landscape of gastric cancer.

Low expression of LBP in gastric cancer cell lines inhibits inflammation-related pathways

To further investigate the role of LBP in the development and progression of gastric cancer, we performed Western blot analysis using AGS and HGC27 gastric cancer cell lines with LBP knockdown. The results demonstrated that LBP protein expression was significantly downregulated in the shLBP group compared to the MOCK and scramble control groups (Fig. 7). Moreover, the reduced expression of LBP led to decreased levels of P65, P-P65, STAT3, and P-STAT3, suggesting that LBP may promote gastric cancer progression by modulating inflammation, immune response, and apoptosis-related pathways.

Discussion

Gastric cancer is among the malignant tumours exhibiting a high incidence and mortality rate throughout the world. Despite advancements in treatment modalities, the long-term survival rates for patients with gastric cancer remain suboptimal. This scenario can largely be

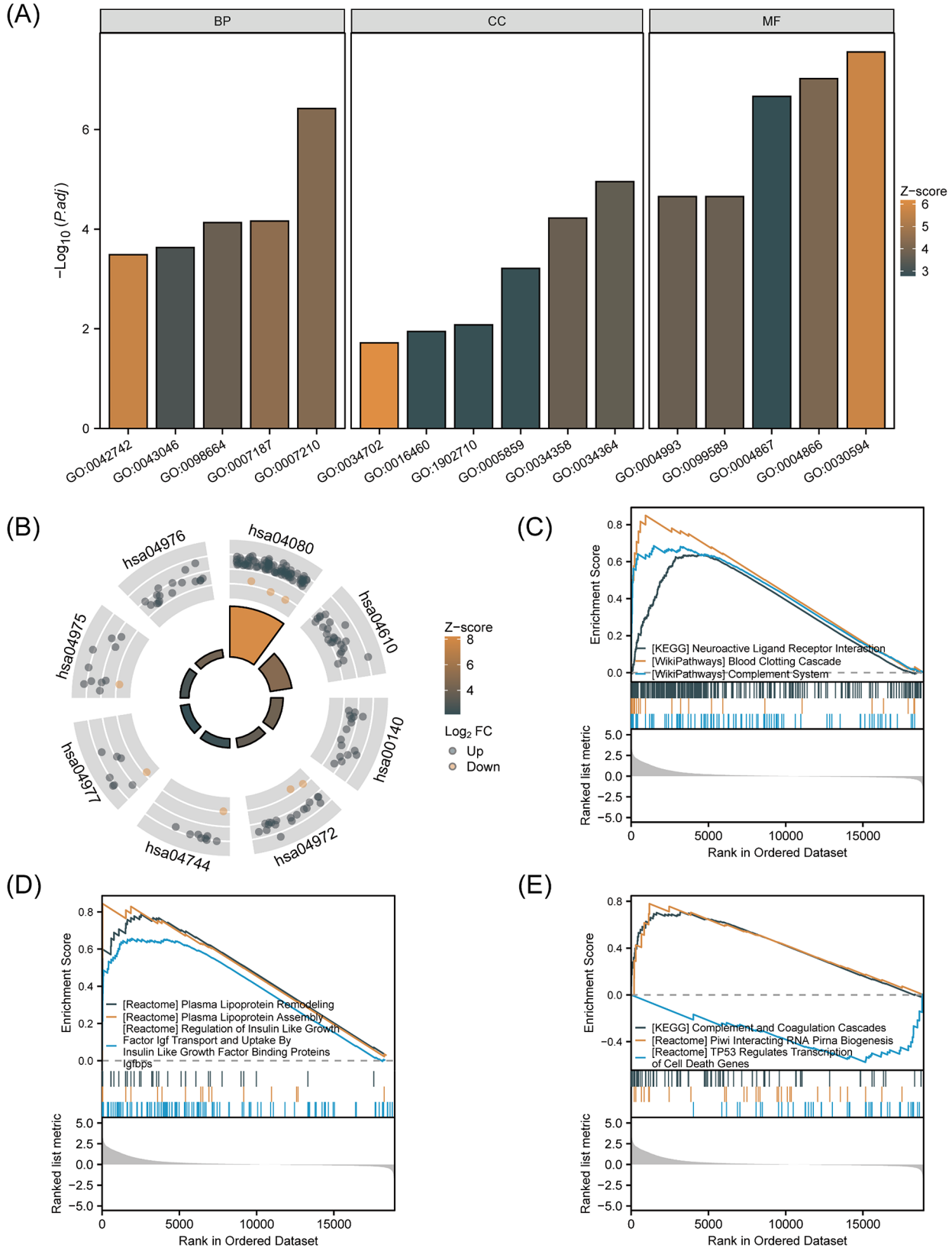

Fig. 5 Functional Enrichment Analysis of LBP and Its Associated Differentially Expressed Genes. (**A**) Results of GO functional enrichment analysis. (**B**) Results of KEGG functional enrichment analysis. (**C**-**E**) Results of GSEA functional enrichment analysis

Table 4 The results of the GO functional enrichment analysis

Ontology	ID	Description	GeneRatio	BgRatio	pvalue	*p*.adjust	zscore
BP	GO:0007210	serotonin receptor signaling pathway	18/1545	40/18,800	5.21e-10	3.81e-07	4.242641
BP	GO:0007187	G protein-coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger	20/1545	67/18,800	2.36e-07	6.9e-05	4.472136
BP	GO:0098664	G protein-coupled serotonin receptor signaling pathway	14/1545	35/18,800	2.69e-07	7.38e-05	3.741657
BP	GO:0043046	DNA methylation involved in gamete generation	10/1545	20/18,800	1.17e-06	0.0002	3.162278
BP	GO:0042742	defense response to bacterium	57/1545	364/18,800	1.7e-06	0.0003	5.695489
CC	GO:0034364	high-density lipoprotein particle	13/1695	27/19,594	9.15e-08	1.11e-05	3.605551
CC	GO:0034358	plasma lipoprotein particle	14/1695	36/19,594	7.62e-07	5.99e-05	3.741657
CC	GO:0005859	muscle myosin complex	8/1695	15/19,594	1.14e-05	0.0006	2.828427
CC	GO:1,902,710	GABA receptor complex	8/1695	21/19,594	0.0002	0.0084	2.828427
CC	GO:0016460	myosin II complex	8/1695	22/19,594	0.0003	0.0113	2.828427
CC	GO:0034702	ion channel complex	42/1695	294/19,594	0.0009	0.0192	6.172134
MF	GO:0030594	neurotransmitter receptor activity	33/1593	111/18,410	1.38e-10	2.77e-08	5.744563
MF	GO:0004866	endopeptidase inhibitor activity	43/1593	180/18,410	5.93e-10	9.54e-08	4.117461
MF	GO:0004867	serine-type endopeptidase inhibitor activity	29/1593	98/18,410	2.01e-09	2.17e-07	2.785430
MF	GO:0004993	G protein-coupled serotonin receptor activity	14/1593	34/18,410	3.3e-07	2.21e-05	3.741657
MF	GO:0099589	serotonin receptor activity	14/1593	34/18,410	3.3e-07	2.21e-05	3.741657

[a] CC, Cellular Component; BP, Biological Process; MF, Molecular Function

Table 5 The results of the KEGG functional enrichment analysis

Ontology	ID	Description	GeneRatio	BgRatio	pvalue	*p*.adjust	zscore
KEGG	hsa04080	Neuroactive ligand-receptor interaction	79/710	362/8164	3.91e-15	5.67e-13	8.213142
KEGG	hsa04610	Complement and coagulation cascades	24/710	85/8164	1.28e-07	1.24e-05	4.898979
KEGG	hsa00140	Steroid hormone biosynthesis	16/710	61/8164	4.41e-05	0.0032	4.000000
KEGG	hsa04972	Pancreatic secretion	20/710	102/8164	0.0004	0.0202	3.577709
KEGG	hsa04744	Phototransduction	9/710	29/8164	0.0006	0.0228	2.333333
KEGG	hsa04977	Vitamin digestion and absorption	8/710	24/8164	0.0007	0.0238	2.121320
KEGG	hsa04975	Fat digestion and absorption	11/710	43/8164	0.0009	0.0277	2.713602
KEGG	hsa04976	Bile secretion	17/710	89/8164	0.0015	0.0434	4.123106

Table 6 The results of the GSEA functional enrichment analysis

ID	Setsize	Enrichmentscore	NES	pvalue	*p*.adjust	qvalue
Kegg_Neuroactive_Ligand_Receptor_Interaction	272	0.6391615	1.329148	4.53e-06	0.0027	0.0026
Wp_Blood_Clotting_Cascade	22	0.8499569	1.603857	4.56e-05	0.0105	0.0100
Wp_Complement_System	96	0.6855782	1.407115	0.0001	0.0187	0.0179
Reactome_Plasma_Lipoprotein_Remodeling	34	0.7874996	1.539967	0.0002	0.0211	0.0201
Reactome_Plasma_Lipoprotein_Assembly	19	0.8441070	1.564729	0.0002	0.0255	0.0243
Reactome_Regulation_Of_Insulin_Like_Growth_Factor_Igf_Transport_And_Uptake_By_Insulin_Like_Growth_Factor_Binding_Proteins_Igfbps	124	0.6573820	1.357477	0.0002	0.0255	0.0243
Kegg_Complement_And_Coagulation_Cascades	69	0.7134234	1.447666	0.0004	0.0315	0.0301
Reactome_Piwi_Interacting_Rna_Pirna_Biogenesis	29	0.7797846	1.506330	0.0005	0.0365	0.0348
Reactome_Tp53_Regulates_Transcription_Of_Cell_Death_Genes	44	-0.5763905	-1.789723	0.0007	0.0410	0.0391

attributed to the complex biological behaviour of gastric cancer, which involves the intricate regulation of numerous molecules and signalling pathways [25]. Our study revealed significant overexpression of LBP in gastric cancer tissues compared with normal gastric tissues, thus suggesting a pivotal role of LBP in the oncogenesis and progression of gastric cancer [26].

LBP is a protein that is closely associated with immune responses, and this protein is capable of recognizing and binding bacterial lipopolysaccharides, thereby activating the host immune system [26]. Our study revealed that elevated expression of LBP in gastric cancer promotes tumour growth and spread. This process may be mediated via the activation of specific signalling pathways, such as the NF-κB and STAT3 pathways, which promote

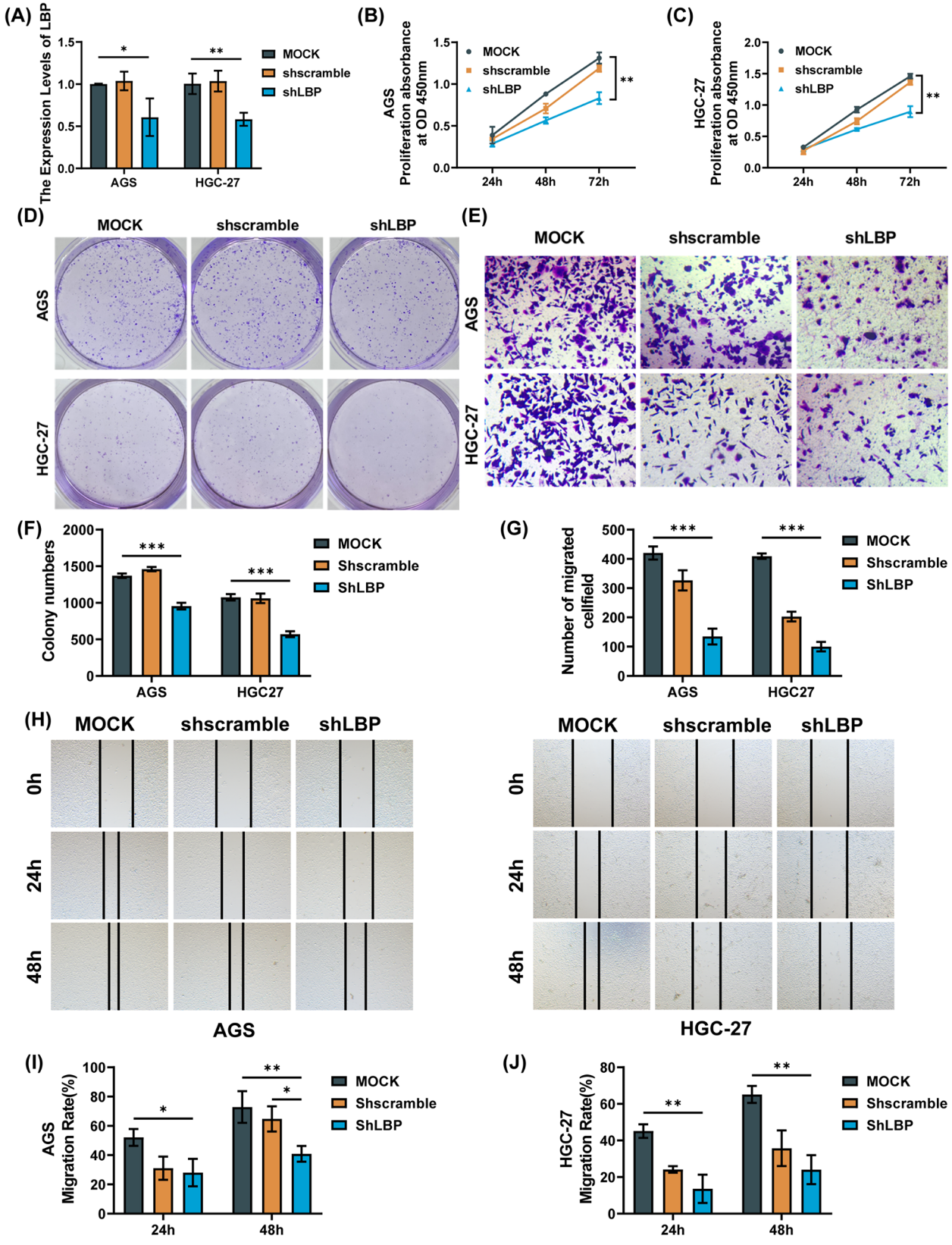

Fig. 6 Effects of LBP Knockdown on Proliferation, Migration, and Invasion in Gastric Cancer Cell Lines. (**A**) qPCR validation of LBP knockdown in gastric cancer cell lines. (**B**, **C**) Results of the CCK-8 assay. (**D**, **F**) Results of the colony formation assay. (**H**-**J**) Scratch wound healing assay results for the AGS and HGC-27 cell lines. (**E**, **G**) Results of the Transwell assay

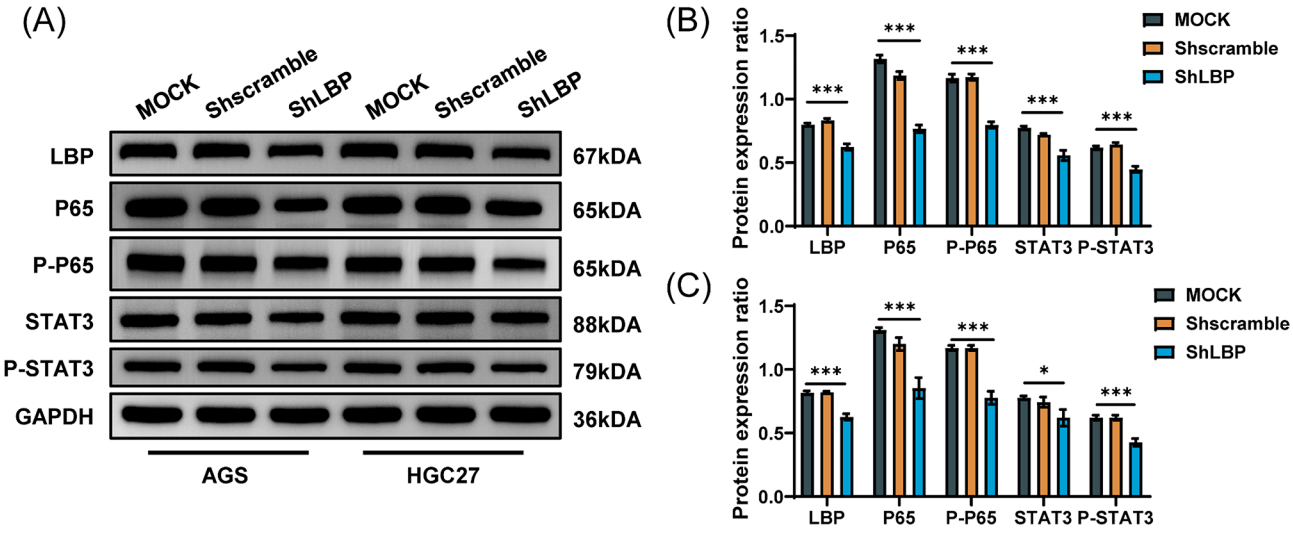

Fig. 7 Effect of LBP Knockdown on the Expression of Inflammation-Related Proteins in Gastric Cancer Cells. (**A**) Expression levels of LBP and related proteins in AGS and HGC27 gastric cancer cell lines. (**B**) Quantitative analysis of protein expression levels in AGS cells. (**C**) Quantitative analysis of protein expression levels in HGC27 cells

inflammatory responses and immune evasion within the tumour microenvironment [27]. Additionally, LBP may directly enhance tumour cell proliferation and survival by modulating the expression of proteins related to the cell cycle and apoptosis [28].

In the development and progression of gastric cancer, the abnormal activation of the NF-κB and STAT3 signalling pathways plays a crucial role. Studies have shown that the expression of NF-κB and STAT3 is significantly greater in gastric cancer tissues than in precancerous lesions and that both factors are activated in gastric cancer cell lines [29].

NF-κB is a key transcription factor involved in cell differentiation, proliferation, apoptosis, and immune responses. In gastric cancer, the persistent activation of NF-κB promotes tumorigenesis and development by inducing chronic inflammation, cellular transformation, and proliferation [30]. Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that participates in various biological processes, including cell proliferation, survival, differentiation, and angiogenesis. In gastric cancer, the abnormal activation of STAT3 is closely associated with tumour proliferation, metastasis, and immune evasion [31].

Moreover, research has shown that in Epstein-Barr virus (EBV)-related gastric cancer, the classical NF-κB signalling pathway remains persistently activated, which possibly occurs via latent infection, thereby promoting gastric cancer development [32].

In summary, LBP promotes the onset and progression of gastric cancer by activating the NF-κB and STAT3 signaling pathways. The aberrant activation of these pathways drives chronic inflammation, cell proliferation, and immune evasion, ultimately facilitating tumor growth and metastasis. Our findings demonstrate that knockdown of LBP in gastric cancer cell lines significantly reduces the expression of key proteins in the NF-κB and STAT3 pathways, further supporting this conclusion. These results highlight the critical role of LBP in gastric cancer progression, particularly through its regulation of NF-κB and STAT3 signaling. Therefore, further investigation into the mechanisms by which LBP modulates these pathways is essential for understanding gastric cancer pathogenesis and developing novel therapeutic strategies.

A comprehensive differential expression analysis of RNA sequencing data from 33 tumour types within TCGA revealed a specific expression pattern of LBP across various cancers [33]. Notably, in gastric cancer, LBP is significantly overexpressed, which is a pattern that is also observed in other cancer types, such as bladder, colorectal, and lung adenocarcinomas. However, its expression is notably decreased in breast cancer and cholangiocarcinoma. Despite the various roles that LBP may play in different cancer types, its generally elevated expression during tumour development and progression, along with its potential diagnostic value in specific cancers, warrant further explorations.

Moreover, our study conducted a prognostic analysis by using clinical and survival information of gastric cancer patients from TCGA, thereby establishing a correlation between LBP expression and patient prognosis. Specifically, high LBP expression was significantly associated with reduced survival rates, which aligns with its role in other types of cancer. For example, in hepatocellular carcinoma, patients with elevated LBP expression exhibited decreased overall survival and shorter recurrence times than did those with low LBP expression [31]. Additionally, a study examining the prognostic potential

of LBP for cardiac function postradiotherapy in patients with breast cancer also correlated LBP levels with cardiac dysfunction, thereby suggesting its utility as a prognostic marker [29]. Although indicative of some predictive value in the short term (one and three years), the area under the ROC curve (AUC) for LBP demonstrated limited long-term prognostic ability [34]. This aligns with findings in oral and maxillofacial tumours, wherein LBP expression is correlated with clinical stage, T classification, and lymph node metastasis [28].

In summary, although the overexpression of LBP in gastric cancer is correlated with both short-term and long-term survival prognoses, its potential as an independent biomarker for long-term survival prediction is limited [35]. These insights necessitate caution when considering the integration of LBP as a clinical prognostic tool, which may potentially require a combination of LBP with other biomarkers or clinical parameters to enhance predictive accuracy [19]. Further investigations into the role of LBP within the tumour microenvironment and its interaction with immune cells could provide novel therapeutic targets and strategies for managing gastric cancer [36].

This study conducted a differential expression analysis of RNA-seq data from gastric cancer patients, thereby stratifying them into groups of high and low LBP expression. We identified 1,895 differentially expressed genes, encompassing 1,791 upregulated and 104 downregulated genes [37]. The elucidation of these genes significantly enhances our understanding of the involvement of LBP in gastric cancer [38]. Using the STRING database for protein-protein interaction (PPI) network analysis, we identified 36 hub genes, including ORM1, RBP4, FGG, SERPINC1, and APOB, all of which have been previously extensively investigated across various cancers [39]. Notably, RBP4 expression in hepatocellular carcinoma has been definitively correlated with patient prognosis; specifically, its decreased expression aligns with advanced disease stages and reduced overall survival rates [40]. Furthermore, variations in the expression of SERPINC1 and AHSG in patient serum following surgical intervention for liver hydatid disease may serve as potential prognostic biomarkers [41]. A thorough analysis of the functional characteristics of these hub genes can help in providing a more detailed understanding of the specific mechanisms and pathways through which LBP may influence gastric cancer progression.

For example, ORM1, which is an acute-phase response protein, plays a major role in regulating immune and inflammatory responses. In gastric cancer, the upregulation of ORM1 likely promotes inflammation in the tumour microenvironment, thereby fostering tumour cell proliferation and migration. The interaction between ORM1 and LBP may synergistically contribute to immune evasion and tumour progression, thereby further emphasizing the role of ORM1 in modulating the tumour immune environment [37].

RBP4 is also closely linked to cancer progression, particularly in gastric cancer. It may contribute to metabolic reprogramming in tumour cells by affecting lipid metabolism and regulating intracellular stress response pathways. This scenario can correspondingly support tumour cell survival and metastasis. The relationship between LBP and RBP4 suggests that these proteins may work together to maintain lipid homeostasis and support tumour cell survival, thus underscoring their potential cooperative role in gastric cancer [28].

APOB is crucial for lipid metabolism and transport, which are vital processes for tumour cell growth and survival. In gastric cancer, APOB may regulate lipid signalling pathways, thereby facilitating tumour cell proliferation and resistance to treatment. The interaction between LBP and APOB further highlights their collaborative role in lipid metabolism and cell survival, thus making them important factors in tumour progression [30].

In addition, genes such as FGG, SERPINC1, SERPINF2, F2, and PLG, which are involved in regulating blood coagulation and anticoagulation, can contribute to tumour invasiveness and metastatic potential by altering the tumour microenvironment and facilitating tumour cell migration. In gastric cancer, FGG has been shown to be correlated with tumour invasiveness and metastasis. The interaction of LBP with FGG may enhance this process, thereby providing a pathway for tumour cells to spread via the bloodstream. Further investigations into the interaction between LBP and FGG could lead to the elucidation of novel therapeutic targets to prevent gastric cancer metastasis [39].

SERPINC1 plays a crucial role in coagulation regulation as a natural anticoagulant. Its interaction with LBP may influence the hypercoagulable state that is commonly observed in gastric cancer, thereby potentially promoting tumour cell invasion and metastasis. This mechanism may also be linked to immune evasion, thus warranting further explorations in future studies [42].

Moreover, genes related to metal metabolism, such as HPX, HP, and CP (which are integral to iron and copper homeostasis), play vital roles in cell division and growth, thus influencing tumour cell viability and proliferation [43]. Proteins encoded by genes such as AMBP, AFM, AHSG, and ALB significantly contribute to the stability of the extracellular matrix and mediate cellular interactions, thus impacting the dynamics of the tumour microenvironment and tumour cell behaviour [44].

Furthermore, genes such as SERPINA3, SERPINA6, SERPINA1, SERPIND1, TF, TTR, GC, ITIH1, ITIH2, CRP, VTN, FGB, and KNG1, which are implicated in inflammatory responses and immune regulation, are

likely to play pivotal roles in modulating the activity of immune cells within the tumour microenvironment, thus influencing both the inflammatory response and immune surveillance [45, 46].

Finally, this study highlights genes such as HP, ASGR1, ASGR2, CHI3L2, ORM2, FGA, FGL1, and AMBP, which are heavily involved in lipid, iron, and copper metabolism, as well as inflammatory processes [47–49]. These metabolic pathways are critical for regulating intracellular signalling and maintaining energy balance, which could significantly affect the metabolic status and proliferation of tumour cells [50, 51]. Concurrently, genes such as SNCAIP, BPIFA1, HPR, APOA1, ORM1, CYP21A2, MAGEA9, SERPINA1, APOC3, C1QL2, SERPINA3, SAA2, FGG, AL049839.2, SAA1, C8B, FGB, RBP4, SYCP2L, PLIN2, ITIH2, LGALS12, CFHR4, SERPINA10, PROKR1, NR1H4, and SSC4D are involved in processes related to coagulation, the immune response, cellular interactions, and apoptosis [52–54]. The altered expression of these genes may be associated with the aggressiveness and metastatic potential of the tumour, thereby significantly affecting tumour microenvironment dynamics and the migratory behaviour of tumour cells [55].

The role of these hub genes in gastric cancer is not limited to a single pathway; rather, they interact through a complex network, thereby collectively regulating tumour cell growth, migration, and immune evasion. Future studies could utilize more refined bioinformatics methods to further reveal the complex relationships between LBP and these hub genes, with a particular focus on their interactions within the tumour microenvironment, which can provide new insights for therapeutic interventions.

Studies have shown that LBP not only plays a key role in immune responses but also significantly affects the regulation of immune cell infiltration in the tumour microenvironment (TME) [56]. By interacting with immune cells in the TME, LBP promotes the establishment of immune evasion mechanisms. Specifically, LBP plays a critical role in the recruitment and polarization of regulatory T cells (Tregs) and tumour-associated macrophages (TAMs) [57].

These findings suggest that LBP may regulate immune cell function and promote immune evasion by activating the TLR4 signalling pathway and its downstream NF-κB and STAT3 pathways. In the gastric cancer tumour microenvironment, LBP may promote the recruitment of Tregs via these pathways. The accumulation of Tregs inhibits effector T-cell function, thereby assisting tumours in evading immune surveillance. Furthermore, LBP may enhance Treg expansion by increasing the secretion of TGF-β1, thus strengthening its immunosuppressive effects, which provides a new mechanistic explanation for immune evasion [58].

LBP may also promote tumour growth and metastasis by influencing the polarization of TAMs. Studies have shown that LBP activates the NF-κB and STAT3 pathways to recruit TAMs, thereby potentially inducing their polarization towards the M2 phenotype. M2-type TAMs secrete immunosuppressive factors such as IL-10 and TGF-β, which inhibit the activity of effector immune cells and further assist tumours in evading immune surveillance. These findings provide theoretical support for the role of LBP in regulating TAM polarization, although this mechanism still requires further validation [56].

Moreover, LBP may influence immune evasion mechanisms through its relationship with immune checkpoints. By modulating immune cell function, LBP may indirectly regulate the expression of immune checkpoint molecules such as PD-1 and CTLA-4, thus enhancing their immunosuppressive effects. Although there is no direct evidence for this phenomenon, existing research suggests that the role of LBP in the immune microenvironment may be closely related to the expression of immune checkpoints [59].

Overall, although the exact mechanisms by which LBP affects immune cell polarization and immune evasion require further investigation, the literature provides strong support for this hypothesis [60]. Future studies should more thoroughly investigate the interactions among LBP, Tregs, TAMs, and immune checkpoints to gain a more comprehensive understanding of the critical role of LBP in immune evasion in gastric cancer.

Functional enrichment analyses revealed that LBP is significantly linked to multiple biological processes, including the serotonin receptor and cyclic nucleotide second messenger-associated G-protein coupled receptor signalling pathways. LBP is instrumental in mediating neuroactive ligand-receptor interactions, complement and coagulation cascades, and steroid hormone biosynthesis [61]. Additionally, it is intimately associated with pathways regulating neurosystem signal transduction, blood coagulation, and immune responses, thus highlighting its pivotal role in these processes [62]. These findings suggest the latent capabilities of LBP in nervous system signalling, immune modulation, lipid metabolism, and gene regulation, thereby offering essential insights for in-depth research into its role in gastric cancer pathogenesis, as well as vital information for new diagnostic and therapeutic strategies [63].

To assess the potential of LBP as a therapeutic target in gastric cancer, we employed shRNA technology to achieve targeted knockdown of LBP expression in the AGS and HGC-27 gastric cancer cell lines [64]. Quantitative PCR confirmed a significant reduction in LBP levels, thus providing a foundation for detailed functional studies. Subsequent assays demonstrated that decreased LBP expression substantially inhibited the proliferative

capacity of these cells, as confirmed via CCK-8 proliferation and colony formation assays. Moreover, scratch healing and Transwell invasion assays indicated that LBP knockdown significantly decreased the migratory and invasive potential of gastric cancer cells.

Given the involvement of LBP in gastric cancer, the targeting of this protein could emerge as being a novel therapeutic strategy. The use of LBP inhibitors or small-molecule agents targeting LBP may inhibit the signalling pathways activated by LBP, thus reducing tumour cell proliferation and enhancing survival, which can ultimately mitigate gastric cancer progression [65]. Furthermore, when considering the link between LBP and immune cell infiltration, the combination of LBP-targeted therapies with immune checkpoint inhibitors may provide a more efficacious treatment approach for gastric cancer patients [66]. Nonetheless, the realisation of this potential involves addressing several challenges, including the precise targeting of LBP and overcoming the immunosuppressive dynamics within the TME.

Conclusion

In conclusion, this study highlights the critical role of LBP in the pathogenesis and progression of gastric cancer. These findings suggest that the high expression of LBP is closely associated with poor prognosis in patients with gastric cancer and is involved in key biological processes, such as immune response regulation and lipid metabolism. By influencing essential processes such as cell proliferation, migration, and invasion, LBP demonstrates significant potential as a diagnostic and prognostic biomarker for gastric cancer.

The role of LBP extends beyond its typical immune functions, with additional functions involving central signalling pathways that promote the invasive behaviour of gastric cancer. The differential expression of LBP in gastric cancer tissues compared with normal tissues, along with its correlation with patient survival outcomes, underscores its potential as a therapeutic target. Notably, the manipulation of LBP expression in gastric cancer cell lines resulted in significant changes in tumour cell dynamics, thus further confirming the viability of LBP as a target for new therapeutic strategies.

Although the results of the present study provide preliminary evidence of the role of LBP in gastric cancer via RNA-seq data, the use of bioinformatics analysis is relatively rare. Future research will need to adopt more refined and complex methods to further elucidate LBP functions within the tumour microenvironment, particularly with respect to immune cell infiltration and immune evasion mechanisms. Advanced techniques such as single-cell RNA sequencing and spatial transcriptomics may reveal more information about the potential role of LBP and therapeutic targets in gastric cancer.

Furthermore, although LBP demonstrates some value in predicting short-term survival, its potential in long-term survival prediction remains to be explored. Future studies could incorporate machine learning and other advanced data analysis methods to validate the predictive effectiveness of LBP, thereby improving the robustness and accuracy of the models. Machine learning techniques may help researchers in better understanding the complex relationship between LBP and gastric cancer, thus providing more precise support for clinical decision-making.

Further research on the role of LBP within the tumour microenvironment (particularly regarding its impact on immune evasion) may reveal new therapeutic targets for existing treatments, including immunotherapy. Overall, this study provides new insights into the molecular mechanisms of gastric cancer, which provides the foundation for translating these findings into clinical practice to improve the management and prognosis of patients with gastric cancer.

In summary, this study suggests that the targeting of LBP may be a novel approach for treating gastric cancer. These findings emphasize the need for further research into the molecular mechanisms of LBP, its interactions within the tumour immune microenvironment, and the integration of more advanced technologies for comprehensive analysis. These efforts will help to advance precision treatment strategies for gastric cancer, thereby providing theoretical support for future clinical research.

Abbreviations

LBP	Lipopolysaccharide-binding Protein
TCGA	The Cancer Genome Atlas
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
HPA	Human Protein Atlas
GSEA	Gene Set Enrichment Analysis
GEO	Gene Expression Omnibus
EMR	Endoscopic Mucosal Resection
CTCs	Circulating Tumor Cells
CB1R	Cannabinoid Type 1 Receptor
GM130	Golgi matrix protein 130
TILs	Tumour Infiltrating Lymphocytes
DCs	Dendritic Cells
LPS	Lipopolysaccharides
BPI	Bacterial Permea-ability-increasing Protein
TNF	Tumor Necrosis Factor
TLR	Toll-Like Receptors
OS	Overall Survival
PFI	Progression Free Interval
DSS	Disease-Specific Survival
PPI	Protein-Protein Interaction Networks

Acknowledgements

Not applicable.

Author contributions

D.L.conceived and designed the Study. H. L. and X. Z.acquired the data and performed the statistical analysis. X.G. and H. L.performed the experiments and analysed the data. H. L.,M.Land X.G.drafted the manuscript. M.L, Y.C.and H. L. contributed to revising the manuscript for intellectual content and language editing.

Funding
None.

Data availability
All data generated or analyzed during this Study are included in this published article.

Declarations

Ethical approval
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Gastroenterology Medicine (Endoscopy Center), China-Japan, Union Hospital of Jilin University, Changchun, Jilin 130033, PR China
[2]Department of Gastroenterology, University of Health and Rehabilitation Sciences Affiliated, Qingdao Municipal Hospital Jiaozhou Road 1#, Qingdao 266071, PR China

Received: 6 November 2024 / Accepted: 17 March 2025
Published online: 28 March 2025

References

1. Amrani Hassani Joutei H, Mahfoud W, Sadaoui I, Fechtali T, Benomar H. [Study of epidemiological clinical and pathological characteristics of gastric adenocarcinoma in a Moroccan population]. Annales de pathologie; 2020.
2. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024;74(1):12–49.
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin. 2021;71(1):7–33.
4. Schumann RR. Old and new findings on lipopolysaccharide-binding protein: a soluble pattern-recognition molecule. Biochem Soc Trans. 2011;39 4:989–93.
5. Ding P-H, Jin L. The role of lipopolysaccharide-binding protein in innate immunity: a revisit and its relevance to oral/periodontal health. J Periodontal Res. 2014;49(1):1–9.
6. Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu Rev Immunol. 1995;13:437–57.
7. Lepper PM, Kleber ME, Grammer TB, Hoffmann K, Dietz S, Winkelmann BR, Boehm BO, März W. Lipopolysaccharide-binding protein (LBP) is associated with total and cardiovascular mortality in individuals with or without stable coronary artery disease–results from the Ludwigshafen risk and cardiovascular health study (LURIC). Atherosclerosis. 2011;219 1:291–7.
8. Zweigner J, Schumann RR, Weber JR. The role of lipopolysaccharide-binding protein in modulating the innate immune response. Microbes Infect. 2006;8 3:946–52.
9. Almeida J, Polvorosa MA, Gonzalez-Quintela A, Marcos M, Pastor I, Hernandez Cerceño ML, Orfao A, Laso FJ. Decreased peripheral blood CD4+/CD25 + regulatory T cells in patients with alcoholic hepatitis. Alcohol Clin Exp Res. 2013;37(8):1361–9.
10. Turgunov Y, Ogizbayeva A, Akhmaltdinova L, Shakeyev K. Lipopolysaccharide-binding protein as a risk factor for development of infectious and inflammatory postsurgical complications in colorectal cancer paients. Contemp Oncol (Pozn). 2021;25(3):198–203.
11. Turgunov Y, Ogizbayeva A, Shakeyev K, Mugazov M, Akhmaltdinova L, Nuraly S, Rudolf V. The dynamics of the lipopolysaccharide-binding protein (LBP) level in assessing the risk of adverse outcomes in operated colorectal cancer patients. Asian J Surg. 2024;47(8):3435–41.
12. Citronberg JS, Curtis KR, White E, Newcomb PA, Newton K, Atkinson C, Song X, Lampe JW, Hullar MA. Association of gut microbial communities with plasma lipopolysaccharide-binding protein (LBP) in premenopausal women. Isme J. 2018;12(7):1631–41.
13. Boylan KL, Andersen JD, Anderson LB, Higgins L, Skubitz AP. Quantitative proteomic analysis by iTRAQ(R) for the identification of candidate biomarkers in ovarian cancer serum. Proteome Sci. 2010;8:31.
14. Chalubinska-Fendler J, Fendler W, Spych M, Wyka K, Luniewska-Bury J, Fijuth J. Lipopolysaccharide-binding protein is efficient in biodosimetry during radiotherapy of lung cancer. Biomed Rep. 2016;5(4):450–4.
15. Cao X, Hu Z, Sheng X, Sun Z, Yang L, Shu H, Liu X, Yan G, Zhang L, Liu C, et al. Glyco-signatures in patients with advanced lung cancer during anti-PD-1/PD-L1 immunotherapy. Acta Biochim Biophys Sin (Shanghai). 2024;56(8):1099–107.
16. Castaño-Rodríguez N, Kaakoush NO, Pardo AL, Goh KL, Fock KM, Mitchell HM. Genetic polymorphisms in the Toll-like receptor signalling pathway in Helicobacter pylori infection and related gastric cancer. Hum Immunol. 2014;75(8):808–15.
17. Chen Q, Du X, Ruan P, Ye Y, Zheng J, Hu S. Bioinformatics Analysis Revealing the Correlation between NF-κB Signaling Pathway and Immune Infiltration in Gastric Cancer. Comput Math Methods Med 2022, 2022:5385456.
18. He Y, Song H, Jiang Y, Ren W. Identification of Immune-Related Prognostic Markers in Gastric Cancer. J Healthc Eng 2022, 2022:7897274.
19. Xie L, Qiu S, Lu C, Gu C, Wang J, Lv J, Fang L, Chen Z, Li Y, Jiang T, et al. Gastric cancer-derived LBP promotes liver metastasis by driving intrahepatic fibrotic pre-metastatic niche formation. J Exp Clin Cancer Res. 2023;42(1):258.
20. Vivian J, Rao AA, Nothaft FA, Ketchum C, Armstrong J, Novak A, Pfeil J, Narkizian J, Deran AD, Musselman-Brown A, et al. Toil enables reproducible, open source, big biomedical data analyses. Nat Biotechnol. 2017;35(4):314–6.
21. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.
22. Liu J, Lichtenberg T, Hoadley KA, Poisson LM, Lazar AJ, Cherniack AD, Kovatich AJ, Benz CC, Levine DA, Lee AV et al. An integrated TCGA Pan-Cancer clinical data resource to drive High-Quality survival outcome analytics. Cell 2018, 173(2).
23. Love MI, Huber W, Anders S. Moderated Estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
24. Jensen LJ, Kuhn M, Stark M, Chaffron S, Creevey C, Muller J, Doerks T, Julien P, Roth A, Simonovic M, et al. STRING 8–a global view on proteins and their functional interactions in 630 organisms. Nucleic Acids Res. 2009;37(Database issue):D412–6.
25. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49.
26. Meng L, Song Z, Liu A, Dahmen U, Yang X, Fang H. Effects of Lipopolysaccharide-Binding protein (LBP) single nucleotide polymorphism (SNP) in infections, inflammatory diseases, metabolic disorders and cancers. Front Immunol. 2021;12:681810.
27. Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. Signal Transduct Target Ther. 2020;5(1):209.
28. Sokolova O, Naumann M. NF-κB signaling in gastric cancer. Toxins (Basel) 2017, 9(4).
29. Kuzmich NN, Sivak KV, Chubarev VN, Porozov YB, Savateeva-Lyubimova TN, Peri F. TLR4 signaling pathway modulators as potential therapeutics in inflammation and sepsis. Vaccines (Basel) 2017, 5(4).
30. Zhang J, Jiang N, Ping J, Xu L. TGF–β1–induced autophagy activates hepatic stellate cells via the ERK and JNK signaling pathways. Int J Mol Med. 2021;47(1):256–66.
31. Luo J, Chen XQ, Li P. The role of TGF-β and its receptors in Gastrointestinal cancers. Transl Oncol. 2019;12(3):475–84.
32. Tsao SW, Tsang CM, Lo KW. Epstein-Barr virus infection and nasopharyngeal carcinoma. Philos Trans R Soc Lond B Biol Sci 2017, 372(1732).
33. Yang L, Ying X, Liu S, Lyu G, Xu Z, Zhang X, Li H, Li Q, Wang N, Ji J. Gastric cancer: epidemiology, risk factors and prevention strategies. Chin J Cancer Res. 2020;32(6):695–704.
34. Xia L, Tan S, Zhou Y, Lin J, Wang H, Oyang L, Tian Y, Liu L, Su M, Wang H, et al. Role of the NFκB-signaling pathway in cancer. Onco Targets Ther. 2018;11:2063–73.
35. Gu Z, Li Y, Yang X, Yu M, Chen Z, Zhao C, Chen L, Wang L. Overexpression of CLC-3 is regulated by XRCC5 and is a poor prognostic biomarker for gastric cancer. J Hematol Oncol. 2018;11(1):115.
36. Nakazawa N, Yokobori T, Sohda M, Hosoi N, Watanabe T, Shimoda Y, Ide M, Sano A, Sakai M, Erkhem-Ochir B et al. Significance of lipopolysaccharides in

gastric cancer and their potential as a biomarker for nivolumab sensitivity. Int J Mol Sci 2023, 24(14).
37. Huang S, Ma L, Lan B, Liu N, Nong W, Huang Z. Comprehensive analysis of prognostic genes in gastric cancer. Aging. 2021;13(20):23637–51.
38. Aimaiti Y, Yusufukadier M, Li W, Tuerhongjiang T, Shadike A, Meiheriayi A, Gulisitan, Abudusalamu A, Wang H, Tuerganaili A, et al. TGF-β1 signaling activates hepatic stellate cells through Notch pathway. Cytotechnology. 2019;71(5):881–91.
39. Chong X, Peng R, Sun Y, Zhang L, Zhang Z. Identification of Key Genes in Gastric Cancer by Bioinformatics Analysis. *Biomed Res Int* 2020, 2020:7658230.
40. Li M, Wang Z, Zhu L, Shui Y, Zhang S, Guo W. Down-regulation of RBP4 indicates a poor prognosis and correlates with immune cell infiltration in hepatocellular carcinoma. Biosci Rep 2021, 41(4).
41. Xu D, Wu J, Dong L, Luo W, Li L, Tang D, Liu J. Serpinc1 acts as a tumor suppressor in hepatocellular carcinoma through inducing apoptosis and blocking macrophage polarization in an Ubiquitin-Proteasome manner. Front Oncol. 2021;11:738607.
42. Wang S, Wang H, Wang K, Zhang Q, Song X. Circulating Exosomal protein EFEMP1 and SERPINC1 as diagnostic biomarkers for epithelial ovarian cancer. Transl Oncol. 2024;50:102126.
43. Lelièvre P, Sancey L, Coll JL, Deniaud A, Busser B. The multifaceted roles of copper in cancer: A trace metal element with dysregulated metabolism, but also a target or a bullet for therapy. Cancers (Basel) 2020, 12(12).
44. Turski ML, Thiele DJ. New roles for copper metabolism in cell proliferation, signaling, and disease. J Biol Chem. 2009;284(2):717–21.
45. Cobine PA, Brady DC. Cuproptosis: cellular and molecular mechanisms underlying copper-induced cell death. Mol Cell. 2022;82(10):1786–7.
46. Wang Z, Jin D, Zhou S, Dong N, Ji Y, An P, Wang J, Luo Y, Luo J. Regulatory roles of copper metabolism and Cuproptosis in human cancers. Front Oncol. 2023;13:1123420.
47. Friedrichs WE, Navarijo-Ashbaugh AL, Bowman BH, Yang F. Expression and inflammatory regulation of haptoglobin gene in adipocytes. Biochem Biophys Res Commun. 1995;209(1):250–6.
48. Levy AP, Levy JE, Kalet-Litman S, Miller-Lotan R, Levy NS, Asaf R, Guetta J, Yang C, Purushothaman KR, Fuster V, et al. Haptoglobin genotype is a determinant of iron, lipid peroxidation, and macrophage accumulation in the atherosclerotic plaque. Arterioscler Thromb Vasc Biol. 2007;27(1):134–40.
49. Prieto-Alamo MJ, Abril N, Osuna-Jiménez I, Pueyo C. Solea senegalensis genes responding to lipopolysaccharide and copper sulphate challenges: large-scale identification by suppression subtractive hybridization and absolute quantification of transcriptional profiles by real-time RT-PCR. Aquat Toxicol. 2009;91(4):312–9.
50. Rocha VZ, Folco EJ, Sukhova G, Shimizu K, Gotsman I, Vernon AH, Libby P. Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. Circ Res. 2008;103(5):467–76.
51. Hirsch HA, Iliopoulos D, Joshi A, Zhang Y, Jaeger SA, Bulyk M, Tsichlis PN, Shirley Liu X, Struhl K. A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. Cancer Cell. 2010;17(4):348–61.
52. Yang B, O'Herrin SM, Wu J, Reagan-Shaw S, Ma Y, Bhat KMR, Gravekamp C, Setaluri V, Peters N, Hoffmann FM et al. MAGE-A, mMage-b, and MAGE-C Proteins Form Complexes with KAP1 and Suppress p53-Dependent Apoptosis in MAGE-Positive Cell Lines. Cancer Research 2007, 67(20):9954–9962.
53. Zhang Y, He J, Zhao J, Xu M, Lou D, Tso P, Li Z, Li X. Effect of ApoA4 on SERPINA3 mediated by nuclear receptors NR4A1 and NR1D1 in hepatocytes. Biochem Biophys Res Commun. 2017;487(2):327–32.
54. Saferali A, Obeidat Me, Bérubé JC, Lamontagne M, Bossé Y, Laviolette M, Hao K, Nickle DC, Timens W, Sin DD, et al. Polymorphisms associated with expression of BPIFA1/BPIFB1 and lung disease severity in cystic fibrosis. Am J Respir Cell Mol Biol. 2015;53 5:607–14.
55. Maslakova AA, Telkov MV, Orlovsky IV, Sokolova OS. Comparative analysis of SERPINA1 gene expression in tumor cell lines. Mosc Univ Biol Sci Bull. 2015;70:127–31.
56. Suzuki M, Mimuro H, Kiga K, Fukumatsu M, Ishijima N, Morikawa H, Nagai S, Koyasu S, Gilman RH, Kersulyte D, et al. Helicobacter pylori CagA phosphorylation-independent function in epithelial proliferation and inflammation. Cell Host Microbe. 2009;5 1:23–34.
57. Kwon Y, Kim M, Kim Y, Jeong MS, Jung HS, Jeoung D. EGR3-HDAC6-IL-27 axis mediates allergic inflammation and is necessary for tumorigenic potential of cancer cells enhanced by allergic inflammation-Promoted cellular interactions. Front Immunol 2021, 12.
58. Interferon-. (cid:1), a Th1 cytokine, regulates fat inflammation A role for adaptive immunity in obesity. In.
59. Thompson ED, Zahurak M, Murphy AG, Cornish TC, Cuka NS, Abdelfatah E, Yang S, Duncan MD, Ahuja N, Taube JM, et al. Patterns of PD-L1 expression and CD8 T cell infiltration in gastric adenocarcinomas and associated immune stroma. Gut. 2016;66:794–801.
60. Ren F, Zhao Q, Minghai Z, Shaogong Z, Liu B-y, Bukhari I, Zhang K, Wu W-Y, Yuming F, Yu Y, et al. Immune infiltration profiling in gastric cancer and their clinical implications. Cancer Sci. 2021;112:3569–84.
61. Noda M, Higashida H, Aoki S, Wada K. Multiple signal transduction pathways mediated by 5-HT receptors. Mol Neurobiol. 2004;29:31–9.
62. Gresch PJ, sanders-Bush E. Serotonin Receptor Signaling. In: 2004; 2004.
63. Freissmuth M, Casey PJ, Gilman AG. G proteins control diverse pathways of transmembrane signaling 1. FASEB J. 1989;3:2125–31.
64. Yang Y-J, Luo S, Wang L-S. Effects of lncRNA-HEIH on proliferation, apoptosis and invasion of gastric cancer cells. Eur Rev Med Pharmacol Sci. 2020;24 18:9400–7.
65. Sun J, Shen D, Gao Y, Zheng Y, Zhao L, Maa M, Liu H, Chen X. Down-Regulation of USP8 suppresses HER-3 positive gastric cancer cells proliferation. OncoTargets Therapy. 2020;13:7973–84.
66. Cui Y, Xie X, Xing Y, Yuan Z, Wu Q, Wei Y. Feasibility study on plasmatic microRNA-27b-3p as a potential biomarker for early diagnosis of gastric cancer. Tumori. 2015;35:183–9.

Publisher's note