Original Article

Value analysis of *ITLN1* in the diagnostic and prognostic assessment of colorectal cancer

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Background: Colorectal cancer (CRC) remains the leading cause of cancer death worldwide. Less than half of the patients are diagnosed when the cancer is locally advanced. Several studies have shown that intelectin-1 (*ITLN1*) can serve as a key prognostic and therapeutic target for CRC. The purpose of this study was to investigate the clinical value of *ITLN1* in CRC and to analyse its potential as a predictive biomarker for CRC.

Methods: Colon adenocarcinoma (COAD) is the main type of CRC. COAD project in The Cancer Genome Atlas (TCGA) database served as the training cohort, and GSE39582 series in the Gene Expression Omnibus (GEO) database served as the external independent validation cohort. First, the difference in the expression level of *ITLN1* between COAD tissue and normal tissue was analysed, and the results were verified via immunohistochemistry. The relationship between *ITLN1* expression and the prognosis of COAD patients was evaluated via the heatmap and the Kaplan-Meier (KM) curve. The *ITLN1* coexpressed gene set obtained by Pearson correlation analysis was used. The prognostic signatures that were significantly correlated with survival status were screened by Cox and least absolute shrinkage and selection operator (LASSO) regression analyses. Finally, a nomogram related to *ITLN1* was constructed based on the risk score of the prognostic signature and routine clinicopathological variables.

Results: *ITLN1* is significantly underexpressed in tumour tissues and can be used as a valuable tool to distinguish COAD. The high-expression group of *ITLN1* was verified to have a greater survival rate. *ITLN1* is significantly associated with a good prognosis in COAD patients. Six candidate genes (*ITLN1* and *MORC2*, *SH2D7*, *LGALS4*, *ATOH1*, and *NAT2*) were selected for use in the Cox-LASSO regression analysis to calculate the risk score. Finally, a nomogram was constructed with a comprehensive risk score and clinicopathologic factors to successfully predict and verify the 1-year, 3-year, and 5-year survival probability.

Conclusions: Our study established *ITLN1* as an effective tool for CRC screening, diagnosis, and prognostic assessment, provided a basis for further study of the molecular function of *ITLN1*, and provided new insights for the mechanistic exploration and treatment of CRC.

Keywords: Intelectin-1 (*ITLN1*); colorectal cancer (CRC); biomarker; prognostic assessment

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in the world (1), influenced by several variables, including the intestinal flora, gene mutations, and the tumour microenvironment (2-4). The prognosis for patients with advanced CRC has dismal, and the 5-year survival rate of stage IV CRC patients is only 12% (5). However, a considerable proportion of CRC morbidity and mortality can be prevented through access to regular screening, surveillance and high-quality treatment (6). With the increasing demand for diagnostic, treatment and prognostic tools for CRC, various screening methods for early diagnosis have been explored. Advances in molecular biology techniques, such as prognostic and predictive biomarkers, provide an opportunity to improve early detection rates and treatment outcomes for CRC (7). The main categories of CRC biomarkers that have been explored are proteins, DNA (detection of mutations and methylation markers), RNA (mainly microRNAs), volatile organic compounds, and changes in the gut microbiome composition and transfer (8).

Human intelectin-1 (intestinal lectin, also known as *ITLN1*) is a 34-kDa secretory protein (9). *ITLN1* has been reported to play a potential role in carcinogenesis (10,11). Li *et al.* reported that *ITLN1* increased the level of hepatocyte nuclear factor 4α (HNF4α), inhibited the nuclear translocation and transcriptional activity of β-catenin in gastric cancer cells, and significantly associated with increased expression of *ITLN1* and improved prognosis in gastric cancer patients (12). Recent studies have shown that *ITLN1* is often lost in CRC tissues (13), and decreased *ITLN1* expression is an independent indicator of the prognosis of patients with CRC (14) and is significantly associated with prognosis in patients with CRC (15-17). These findings suggest that *ITLN1* has a tumour suppressor effect on gastrointestinal cancers. However, the diagnostic and prognostic value of *ITLN1* in CRC needs to be further evaluated.

The purpose of this study was to investigate the clinical value of *ITLN1* in CRC and to analyse its potential as a predictive biomarker for CRC. First, we used The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) databases to analyse the difference in the expression of *ITLN1* between tumour tissues and normal tissues. Immunohistochemical staining was used for further verification. The relationship between the *ITLN1* expression level and the survival prognosis of colon adenocarcinoma (COAD) patients was also investigated. The gene sets coexpressed with *ITLN1* were identified by correlation analysis. Functional enrichment analysis and Cox-LASSO (least absolute shrinkage and selection operator, LASSO) algorithm dimension reduction analysis were performed on these gene sets, and several prognostic signatures significantly correlated with overall survival (OS) were obtained. Nomograms were constructed by combining the risk score (which was calculated using these prognostic signatures) and clinicopathological factors to predict 1-year, 3-year, and 5-year survival in COAD patients. Finally, independent validation was performed with the GSE39582 validation cohort. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-137/rc).

Highlight box

Key findings

- Intelectin-1 (*ITLN1*) was established as an effective tool for colorectal cancer (CRC) screening, diagnosis, and prognostic assessment. A nomogram was developed and validated based on the *ITLN1* risk score to predict the overall survival of CRC patients.

What is known and what is new?

- High *ITLN1* expression is associated with good prognosis in cancers, including gastric cancer, lung cancer and neuroblastoma, etc.
- This study found that *ITLN1* was significantly underexpressed in CRC tissues and significantly correlated with patient prognosis, suggesting that *ITLN1* could be used as an effective differentiating tool and therapeutic target for CRC. A nomogram based on *ITLN1* risk score and clinicopathological factors was constructed to fully investigate the role of *ITLN1* in CRC progression.

What is the implication, and what should change now?

- The potential of *ITLN1* as a predictive biomarker for CRC is confirmed.
- The efficacy and safety of this nomogram need to be verified by further large-scale clinical trials.

Methods

Data sources and processing

COAD is the main type of CRC (18). The mRNA expression data and relatively complete clinical information (such as age, gender, T classification, N classification, metastasis status, tumour stage, and survival status) of COAD patients in the TCGA training cohort were obtained from the University of California, Santa Cruz

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(UCSC) Xena (https://xena.ucsc.edu/). These included 453 COAD tissue samples (tumour) and 41 normal tissue samples (normal). To independently validate the diagnostic value, prognostic value and clinical value of *ITLN1*, we used the GSE39582 validation cohort from the National Center for Biotechnology Information GEO database (https://www.ncbi.nlm.nih.gov/geo) as an external independent validation cohort. GSE39582 cohort involves 585 patients with stage I to IV CRC who underwent surgery between 1987 and 2007 in seven centers. There were 566 tumour samples and nineteen normal samples. The median follow-up was 51.5 months. The gene expression levels of both the TCGA training cohort and the GSE39582 validation cohort were converted to a log2 scale.

Immunohistochemical staining

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Wannan Medical College [Wuhu, China; ethics approval number: (2023) 215], and written informed consent was obtained from COAD patients to carry out immunohistochemical experiments for *ITLN1* (Experiment date: December 2023). The inclusion criteria for patients were as follows: had CRC (aged 30 to 90 years), male and female half. Exclusion criteria: pregnant women and nursing mothers. The paired CRC tissue and paracancerous tissue samples were obtained from the same subject. Immunohistochemical staining was performed on samples from ten patients diagnosed with CRC who had undergone primary surgery at The Second Affiliated Hospital of Wannan Medical College from August to November 2023, and CRC tumour tissue samples and adjacent tissue samples were collected from each patient. All the samples were completely deidentified before the start of the immunohistochemical staining experiment. Formalin-fixed, paraffin-embedded tissue blocks were subjected to immunohistochemical analysis of *ITLN1* according to the manufacturer's instructions. Briefly, after partial paraffin dewaxing and antigen retrieval with citrate buffer, 3% hydrogen peroxide was used to block endogenous peroxidase activity. After serum closure, the sections were incubated with the primary antibody (Affinity Biosciences, Changzhou, China; Art No: DF12413) at 4 ℃ overnight and with the secondary antibody for two hours, followed by diaminobenzidine (DAB) colour development and haematoxylin reverse staining. Images were taken at ×100 and ×200 magnifications using a German Leica upright microscope. According to the positive intensity of immunostaining, it is divided into 0 points (colorless). 1 point (light yellow), 2 points (brown yellow), 3 points (dark brown); According to the mean percentage of positive tumour cells, the ratio of positive cells to tumour cells was <10%, 10–50%, 50–75%, >75%. They are divided into 1 to 4 points. The percentage of positive tumour cells and staining intensity were multiplied to produce a weighted score: <3 score (–), 3–5 score (+), 6–9 score (++), >9 score (+++), which was double-blind detected by two senior diagnostic physicians (19,20).

Differential expression analysis and Kaplan-Meier (KM) prognosis analysis

In this study, the R package ggplot2 was used for expression analysis, and differences in expression levels were examined between tumour tissues and normal tissues. Moreover, R package pROC was used to analyze the optimal threshold of *ITLN1* expression and the receiver operating characteristic (ROC) curve. The ROC curve evaluated the efficacy of *ITLN1* as a biomarker of COAD, and the area under the curve (AUC) was used as a measure. The median *ITLN1* expression was used as the cut-off for classifying patients into two groups: Patients with *ITLN1* expression higher than the median were defined as the H-*ITLN1* subgroup, and those with *ITLN1* expression lower than the median were defined as the L-*ITLN1* subgroup. KM survival curves were generated to analyse the prognostic value of *ITLN1* in CRC patients.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

The genes coexpressed with *ITLN1* in the TCGA training cohort were identified by Pearson correlation analysis (21). After the correlation coefficient was set to $|r|>0.4$ and $P<0.05$, 344 coexpressed genes were screened. The ClusterProfiler package and David platform (https://david-d.ncifcrf.gov/) were used for GO functional enrichment analysis and KEGG pathway enrichment analysis.

Cox-LASSO dimension reduction analysis

Univariate Cox regression analysis was used to narrow the gene screening scope, *ITLN1* was significantly related to OS in the TCGA COAD cohort, and a forest plot was generated. LASSO dimension reduction analysis was

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performed using the glmnet and survival software packages in R, and the λ value corresponding to the minimum partial likelihood deviation was selected as the optimal λ value in this study (22). Six candidate genes and their corresponding λ values were obtained, and the risk score was ultimately calculated. The formula was developed as follows: risk score = (–0.039986125433698) × Exp(*ITLN1*) + (0.557302748801436) × Exp(*MORC2*) + (0.914314537656243) × Exp(*SH2D7*) + (–0.058343048355548 2) × Exp(*LGALS4*) + (–0.104557483236321) × Exp(*ATOH1*) + (–0.191977190223862) × Exp(*NAT2*).

To screen potentially informative markers significantly related to the survival status of patients with CRC, patients from the TCGA COAD training cohort were divided into high- and low-risk groups according to the median value of the risk score. Combined with the survival status and survival period of the patients, the relationships between the risk score and clinicopathological factors, prognosis and survival were plotted.

A KM survival curve was used to explore differences in survival and prognosis between the two groups. We then used a ROC curve to evaluate the predictive value of the *ITLN1* prognostic signature (23).

Nomogram construction and evaluation

With respect to the TCGA training cohort, a nomogram was constructed by combining the risk score and clinicopathological factors using the rms package. The 1-year, 3-year, and 5-year survival rates could be accurately predicted by the total score and single factor score. To independently verify the accuracy of OS prediction of our established nomogram, calibration curves and C-index were calculated to evaluate the accuracy of the survival prediction. The C-index ranges from 0.5 to 1.0, with 0.5 indicating a random probability and 1.0 indicating a perfect fit. In general, a C-index value greater than 0.65 indicates a reasonable estimate (24). The consistency of the predicted 1-year, 3-year, and 5-year OS with the actual OS is presented by calibration curves. The feasibility of the nomogram was confirmed by external validation using the GSE39582 GEO dataset.

Statistical analysis

All clinical data, including age, gender, OS, tumour stage, T classification, N classification, and metastasis, along with the genetic expression matrix, were statistically analysed using R version 4.2.2 and several R packages like tidyverse, DESeq2, ggplot2 and survminer. An unpaired *t* test was used to determine the significance of differences between the two groups; one-way analysis of variance (ANOVA) was used to compare the differences between three or more groups. To assess the significance of the difference in prognosis between the *ITLN1* high-expression group and the *ITLN1* low-expression group, KM curves were generated using the log-rank test. The P value of Pearson correlation analysis has been corrected by Bonferroni.

Results

Diagnostic value of ITLN1

The level of mRNA expression of *ITLN1* was significantly lower in the tumour tissue samples than in the normal tissue samples (P<0.001) (*Figure 1A*). By performing ROC curve analysis to discriminate tumour tissue from normal tissue samples, we found that the AUC of the *ITLN1* expression level was 0.894 (The optimal threshold of *ITLN1* expression was 6.6), suggesting that *ITLN1* could be a good diagnostic assistance reference tool (*Figure 1B*).

In addition, immunohistochemical staining for *ITLN1* was lower in COAD tumour tissues than in adjacent noncancerous colon tissues, indicating decreased protein expression in COAD (*Figure 1C*). Immunohistochemical scoring is shown in Table S1.

Clinical value and prognostic value of ITLN1

With increasing *ITLN1* expression, the survival status and clinicopathological factors exhibited asymmetric distributions (*Figure 2A*). KM survival analysis revealed that the H-*ITLN1* subgroup was associated with longer OS than the L-*ITLN1* subgroup (P=0.006) (*Figure 2B*).

Moreover, we analysed the relationships between *ITLN1* expression and clinical parameters, including age, gender, stage, metastasis status, N classification, and T classification (*Table 1*). *ITLN1* expression tended to decrease in patients with advanced-stage disease, advanced-T classification, or advanced metastasis, although this trend was not statistically significant.

Functional enrichment analysis of ITLN1 coexpressed genes

In the TCGA training cohort, 344 genes related to *ITLN1*

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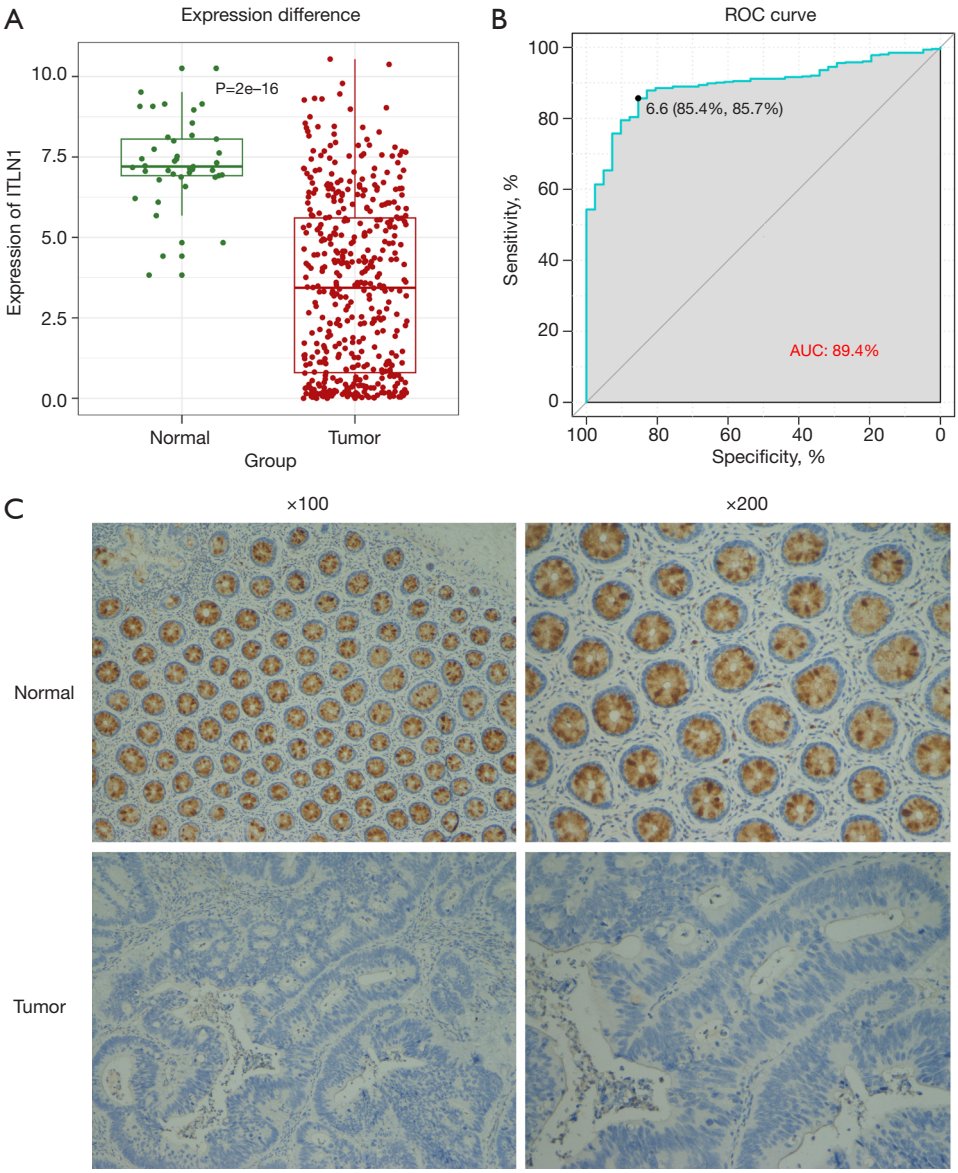

Figure 1 Diagnostic value of *ITLN1*. (A) Comparison of *ITLN1* mRNA expression levels between tumour tissues and normal tissues. (B) Diagnostic efficacy of *ITLN1* according to the ROC curve. (C) The expression level of *ITLN1* in COAD tissues and adjacent noncancerous colon tissues was determined via immunohistochemical staining. ROC, receiver operating characteristic; AUC, area under the curve; COAD, colon adenocarcinoma.

were identified by Pearson correlation analysis. Then, GO and KEGG analyses were performed: biological processes (BP) most related to *ITLN1* included cellular cation homeostasis, hormone metabolic process, primary alcohol metabolic process, oligosaccharide metabolic process, and oligosaccharide biosynthetic process (*Figure 3A*); *ITLN1*'s most related cellular component (CC) was an external side of the apical part of the cell (*Figure 3B*); the molecular function (MF) was glycosyltransferase activity (*Figure 3C*); and *ITLN1*'s most related signalling pathways were metabolic pathway and bile secretion pathway (*Figure 3D*).

The effect of the risk score constructed by Cox-LASSO on the prognosis of COAD patients

Univariate Cox analysis (P<0.05) was performed on 344

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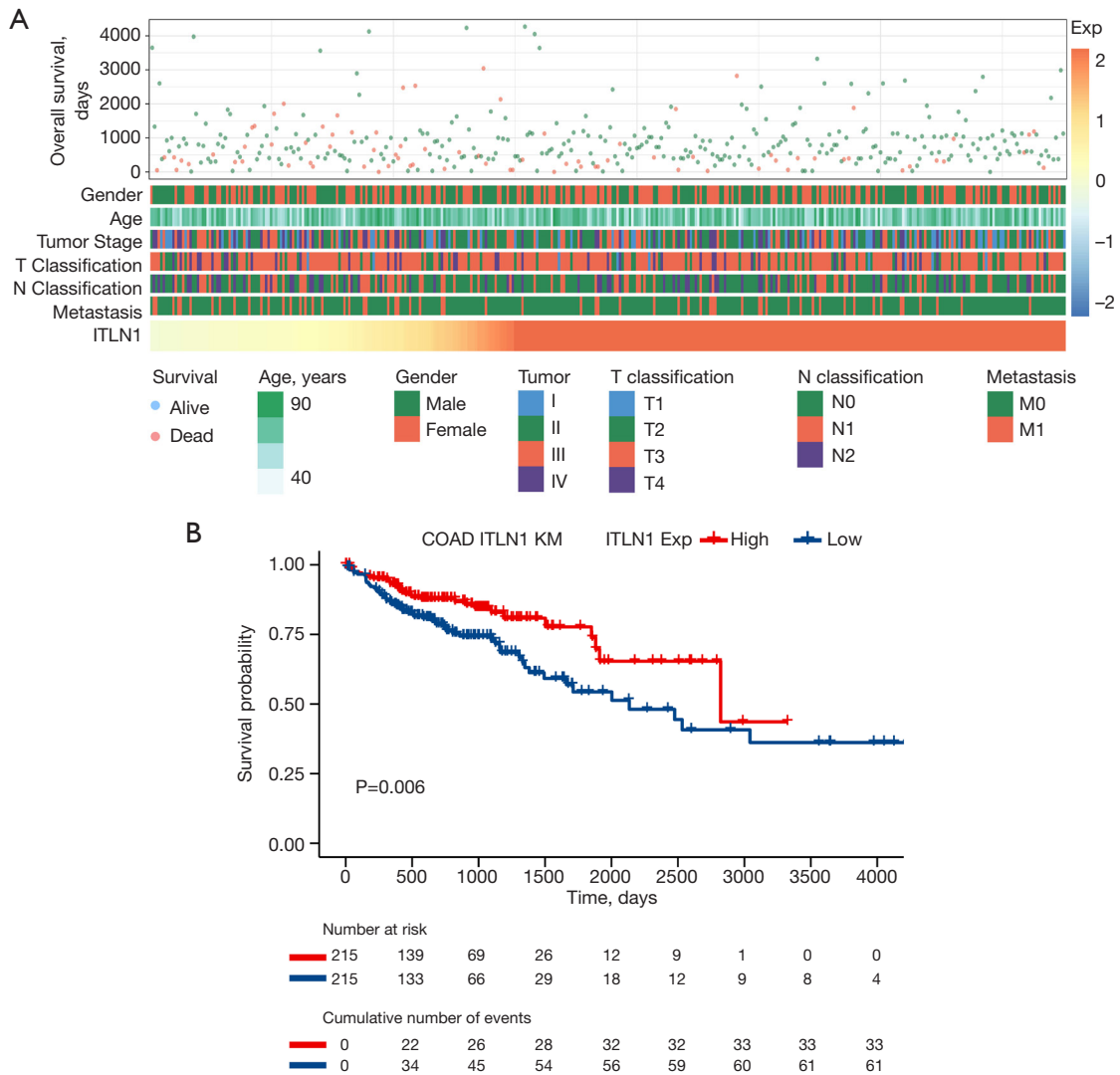

Figure 2 Clinical value and prognostic value of *ITLN1*. (A) As *ITLN1* expression increased, the survival status and clinicopathological factors exhibited asymmetric distributions. (B) Survival analysis was performed to evaluate differences in survival between the high-*ITLN1* and low-*ITLN1* groups. COAD, colon adenocarcinoma; KM, Kaplan-Meier.

genes associated with *ITLN1* to screen out eight genes related to OS (*Figure 4A*). Six genes (*ITLN1*, *SPINK4*, *LGALS4*, *ATOH1*, *CLCA1*, and *NAT2*) had hazard ratio (HR) less than one and were considered good prognostic factors, while the other two genes (*MORC2* and *SH2D7*) had HR greater than one and were considered poor prognostic factors.

Five genes related to *ITLN1* were further identified as prognostic genes by LASSO regression analysis (*Figure 4B,4C*): *MORC2*, *SH2D7*, *LGALS4*, *ATOH1* and *NAT2*. Among them, *SH2D7*, *LGALS4*, *ATOH1* and *NAT2* were positively correlated with *ITLN1*, and *MORC2* was negatively correlated with *ITLN1* (Figure S1). The expression levels of these five genes also significantly differed between the normal group and the tumour group (Figure S2). KM curve analysis revealed that *MORC2*, *LGALS4*, *ATOH1* and *NAT2* were significantly correlated with the survival of COAD patients (Figure S3). Cox-LASSO analysis identified six genes, including *ITLN1*, for calculating the risk score. The median risk score was used as the cut-off value to classify patients into a low-risk group and a high-risk group. Significant differences were observed in the expression of six genes between the two groups of patients

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Table 1 Comparison of clinical parameters between the H-*ITLN1* and L-*ITLN1* groups in COAD patients

Clinical parameters	H-*ITLN1*		L-*ITLN1*	
	Alive (N=200)	Dead (N=46)	Alive (N=179)	Dead (N=67)
Age (years)				
Mean (SD)	66.5 (13.2)	70.1 (13.8)	66.3 (12.8)	70.6 (12.3)
Median [min, max]	67.0 [31.0, 90.0]	74.0 [34.0, 90.0]	68.0 [34.0, 90.0]	73.0 [40.0, 90.0]
Gender				
Female	95 (47.5%)	21 (45.7%)	87 (48.6%)	30 (44.8%)
Male	105 (52.5%)	25 (54.3%)	92 (51.4%)	37 (55.2%)
Stage				
I	37 (18.5%)	4 (8.7%)	36 (20.1%)	2 (3.0%)
II	90 (45.0%)	16 (34.8%)	75 (41.9%)	17 (25.4%)
III	49 (24.5%)	11 (23.9%)	50 (27.9%)	23 (34.3%)
IV	20 (10.0%)	12 (26.1%)	16 (8.9%)	22 (32.8%)
NA	4 (2.0%)	3 (6.5%)	2 (1.1%)	3 (4.5%)
T classification				
T1	5 (2.5%)	1 (2.2%)	4 (2.2%)	1 (1.5%)
T2	39 (19.5%)	5 (10.9%)	36 (20.1%)	2 (3.0%)
T3	140 (70.0%)	30 (65.2%)	119 (66.5%)	48 (71.6%)
T4	15 (7.5%)	10 (21.7%)	20 (11.2%)	16 (23.9%)
NA	1 (0.5%)	0	0	0
N classification				
N0	135 (67.5%)	21 (45.7%)	114 (63.7%)	24 (35.8%)
N1	37 (18.5%)	8 (17.4%)	44 (24.6%)	21 (31.3%)
N2	28 (14.0%)	17 (37.0%)	21 (11.7%)	22 (32.8%)
Metastasis				
M0	157 (78.5%)	24 (52.2%)	141 (78.8%)	36 (53.7%)
M1	20 (10.0%)	12 (26.1%)	16 (8.9%)	22 (32.8%)
NA	23 (11.5%)	10 (21.7%)	22 (12.3%)	9 (13.4%)

COAD, colon adenocarcinoma; SD, standard deviation; NA, not available (represent missing value).

(Figure S4). Moreover, the two groups of patients were sorted in ascending order according to the risk score, and the one-to-one correspondence between the risk score and the survival state and survival period of the patients was analysed in a chart. The results showed that the proportion of patients who died increased significantly with increasing risk score (*Figure 4D*). Subsequently, KM survival curves for patients in different risk score groups (high and low) showed that patients in the high-risk group had a significantly worse prognosis and shorter survival probability (*Figure 4E*). The AUC of the 1-year, 3-year and 5-year time ROC curves were all greater than 65%, indicating a significant difference in survival probability between high-risk and low-risk patients (*Figure 4F*).

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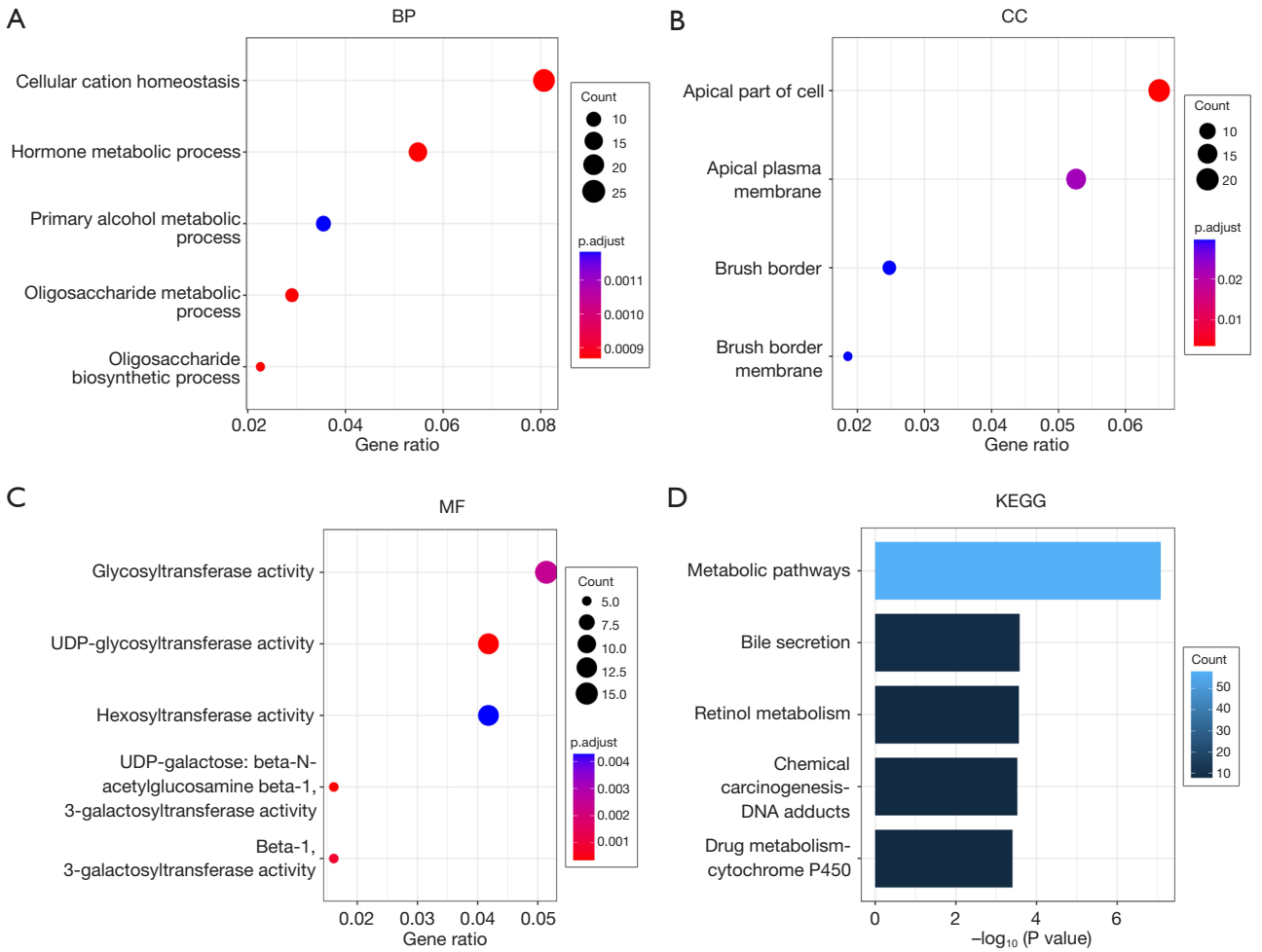

Figure 3 Functional enrichment analyses of *ITLN1* coexpressed genes in CRC. (A-C) BP, CC, and MF terms are mostly related to *ITLN1*. (D) KEGG pathway analysis of *ITLN1*. CRC, colorectal cancer; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Nomogram construction based on the 6-gene risk score and pathological parameters

An individualized prediction model for OS prediction was constructed based on independent predictive factors, including the risk score, T classification, age, and tumour stage. The nomogram showed that the 1-year, 3-year, and 5-year survival probabilities of COAD patients could be estimated by the individualized prediction model (*Figure 5A*). The C-index reached 0.791 in the TCGA training cohort. The overall prediction accuracy was greater than that of a single factor (*Figure 5B*). The nomogram and actual observations in the calibration curve showed satisfactory overlap in the TCGA training cohort (*Figure 5C-5E*) and the GSE39582 validation cohort (*Figure 5F-5H*), indicating optimal predictive accuracy.

Validation based on the GSE39582 validation cohort

The reliability of *ITLN1* as a diagnostic and prognostic marker for CRC was validated in the GSE39582 validation cohort. We found that the expression level of *ITLN1* was effective at distinguishing between tumour and normal tissues; the AUC of the *ITLN1* expression level was 0.91 (The optimal threshold of *ITLN1* expression was 10.2). That is, the expression level of *ITLN1* could be used as a diagnostic factor for COAD (*Figure 6A,6B*). In addition, the median *ITLN1* expression in the database was divided into high and low groups. KM survival analysis revealed that

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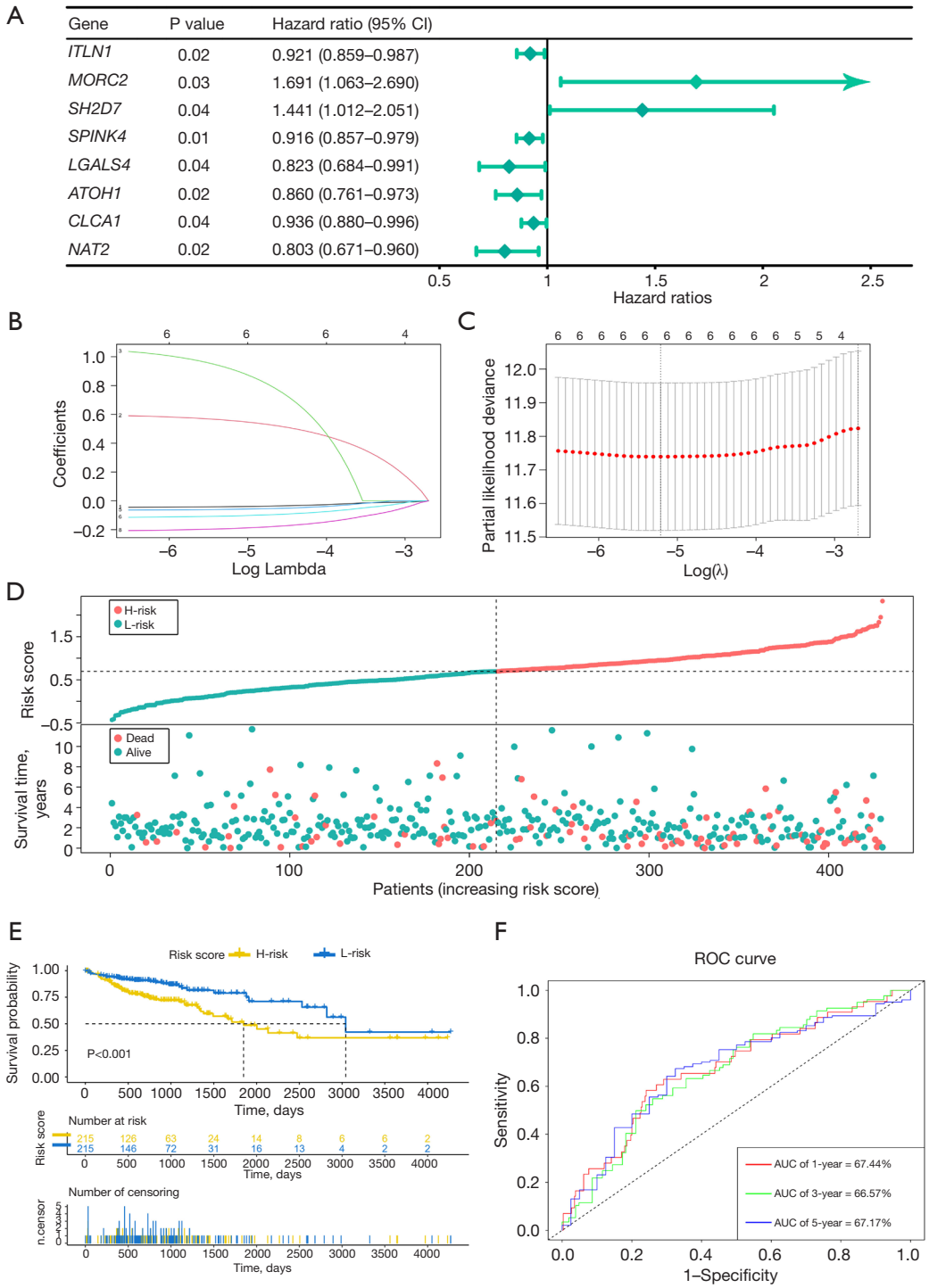

Figure 4 The influence of the risk score on the prognosis of CRC patients was explored. (A) Forest plot of the 8 genes identified by univariate Cox regression analysis. The hazard ratio are death risk ratios, where values greater than 1 are poor prognostic factors and vice versa. (B,C) LASSO coefficient profiles of 6 prognosis-related genes. The red dots represent the partial likelihood values. The optimal parameter (λ) was calculated by tenfold cross-validation. (D) The distribution of the risk score and survival status of CRC patients in the TCGA training cohort. The X-axis here is the number of patients in ascending order of risk score, and the Y-axis is the risk score and survival probability. (E,F) KM survival curves were analysed for different risk assessment groups (H-risk and L-risk), and ROC curves were drawn. CRC, colorectal cancer; CI, confidence interval; LASSO, least absolute shrinkage and selection operator; TCGA, The Cancer Genome Atlas; H, high; L, low; KM, Kaplan-Meier; ROC, receiver operating characteristic; AUC, area under the curve.

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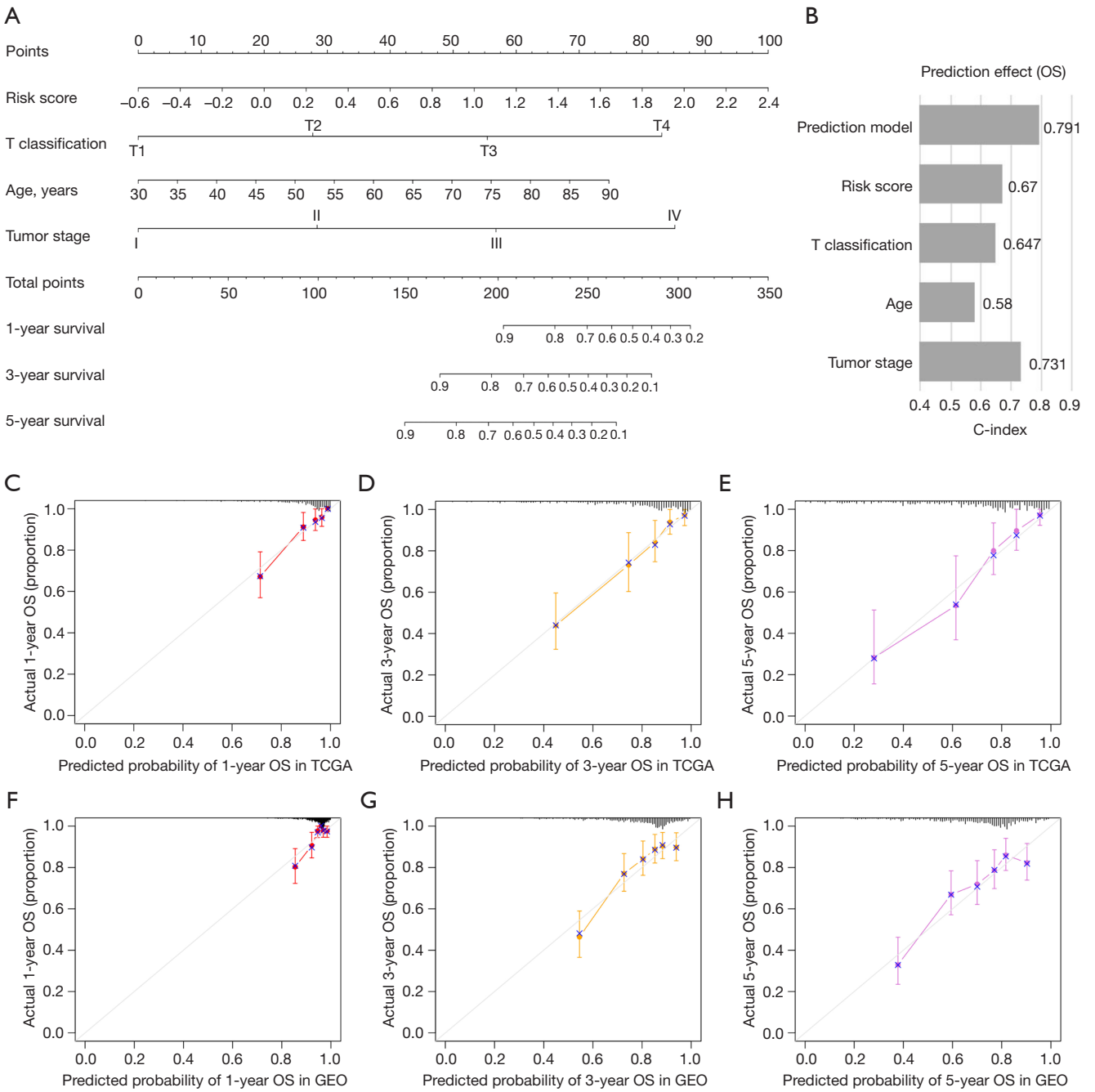

Figure 5 The prognostic value evaluation of the nomogram based on the *ITLN1* risk score in CRC patients. (A) A nomogram was constructed to predict 1-year, 3-year, and 5-year OS by combining the risk score, age, T classification, and tumour stage. (B) The C-index was used to evaluate the ability of the individualized prediction model, risk score, T classification, age and tumour stage to predict OS in patients with CRC. (C-E) Calibration curves of the nomogram for predicting and observing 1-year, 3-year, and 5-year OS in the TCGA training cohort. The dashed line at 45° indicates a perfect prediction. (F-H) Calibration curves of the nomogram for predicting and observing 1-year, 3-year, and 5-year OS in the GSE39582 validation cohort. CRC, colorectal cancer; OS, overall survival; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus.

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survival was significantly greater in the H-*ITLN1* subgroup than in the L-*ITLN1* subgroup (*Figure 6C*). Subsequently, the results of functional enrichment analysis of *ITLN1* were verified, and the results showed that *ITLN1* was still associated mainly in glycosyltransferase activity and other biological functions (*Figure 6D-6G*). These results are consistent with our findings in the TCGA training cohort.

Discussion

One of the prevalent gastrointestinal malignancies, at present, several bioinformatics studies have been conducted to identify biomarkers in CRC, such as *TOP2A*, *MAD2L1*, *CHEK1*, *SST*, *CXCL8*, and *GUCA2A* (25,26). Compared with the findings of recent studies focused on screening differentially expressed genes (DEGs) as potential biomarkers, the clinical and prognostic value of biomarkers and their relationship with survival in patients with CRC have received little attention.

Our study showed that the expression level of *ITLN1* in COAD tissues was significantly lower than that in normal tissues and could be used as a valuable tool to distinguish COAD, which was confirmed by immunohistochemical staining. Moreover, external independent verification was carried out with the GEO database. Low levels of *ITLN1* have been correlated with obesity-related colorectal carcinogenesis (14). Katsuya *et al.*, through quantitative reverse transcription-polymerase chain reaction (RT-PCR), showed that *ITLN1* expression was reduced in more than half of the CRC patients investigated. Reduced *ITLN1* expression was found to be an independent prognostic marker for patients with CRC (16). Furthermore, GO and KEGG enrichment analyses revealed that *ITLN1* was strongly associated with multiple biological functions, including hormone metabolic process, primary alcohol metabolic process and oligosaccharide metabolic process. These biological functions are the hallmarks of cancer (27), suggesting that *ITLN1* plays vital roles in the diagnosis and progression of CRC.

To study the relationship between *ITLN1* and the survival of CRC patients, we analysed the relationship between *ITLN1* expression and CRC stages and found that CRC metastasis occurred more frequently in patients with reduced *ITLN1* expression. The KM survival curve revealed a significant positive correlation between *ITLN1* expression and survival of CRC patients. There is already evidence that *ITLN1* regulates cell proliferation, activation, migration and differentiation by participating in the glucose metabolism pathway, fat metabolism pathway and protein metabolism pathway and inhibiting the occurrence and metastasis of tumours. Moreover, a low expression level of *ITLN1* leads to dysregulation of the PI3K/Akt pathway (28). The PI3K/Akt pathway is an intracellular signalling pathway related to proliferation, differentiation and apoptosis and is an important pathway for body self-protection. When activated, PI3K phosphorylates its downstream molecule Akt, thereby inhibiting apoptosis and regulating cell survival and proliferation (29). In addition, *ITLN1* can reduce the level of secondary bile acid by inhibiting bile secretion in CRC patients, thus achieving cancer inhibition (30). These findings suggest that *ITLN1* plays an inhibitory role in CRC and demonstrate the potential prognostic value of *ITLN1* in CRC. These findings are consistent with our research.

To further evaluate the prognostic value of *ITLN1* in CRC, we calculated the risk score based on the expression level and prognostic value of *ITLN1*. We divided COAD patients into two groups according to the risk score and evaluated the survival of patients with different risk scores. The results showed that the survival of patients with higher risk scores was significantly shorter. There is evidence that *ITLN1* can reduce the malignant behaviour of CRC cells, as indicated by cell growth, metastasis and invasion, and that decreased *ITLN1* expression is independently associated with the progression and poor prognosis of CRC (16). Kim *et al.* identified *ITLN1* as a marker of favourable outcome in stage IV CRC patients (17). Thus, *ITLN1* may be a potential predictive biomarker for survival in CRC patients.

Given these preliminary findings, a nomogram was constructed based on the risk score and clinicopathological factors to predict survival in COAD patients. We found that tumour stage was the most sensitive predictor, and a previous study has confirmed this (31). Therefore, in this study, all stages of CRC patients were selected. Moreover, the T classification was one of the important factors affecting the prognosis. On the basis of existing reports, scholars agree that, compared with other prediction models, a greater T classification increase indicates that disease deterioration substantially affects survival and has been widely used in various cancer prediction models (32,33). In this study, we constructed a nomogram based on the risk score (which was obtained from *ITLN1* expression and prognostic value), tumour stage, T stage, and age, which has better accuracy than any single clinical factor prediction model. At present, there are many published nomograms for predicting the prognosis of CRC patients. Wang *et al.* recently proposed for the first time the use of metastasis-

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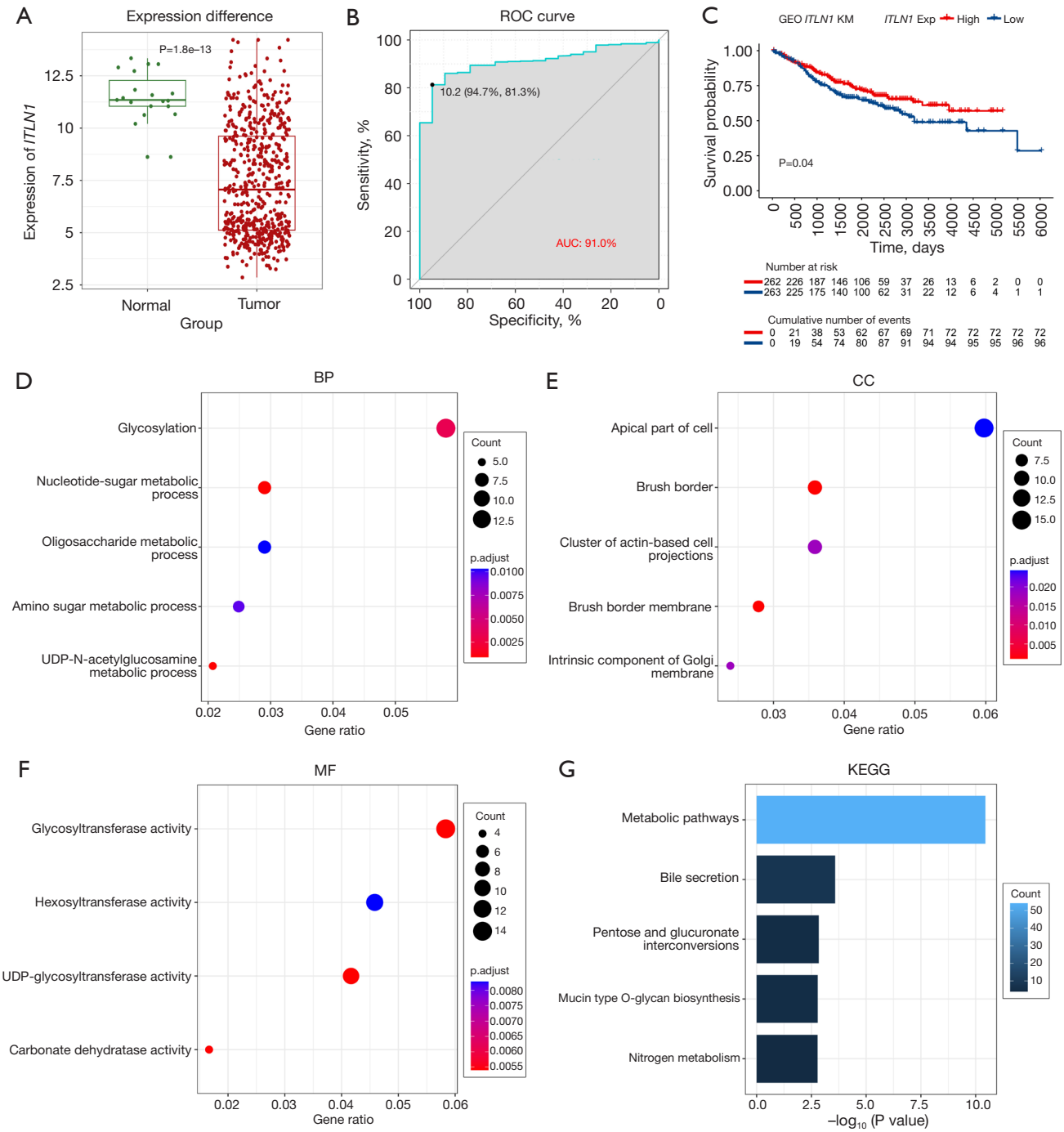

Figure 6 The diagnostic value, prognostic value and functional enrichment of *ITLN1* in CRC were verified in the GEO dataset GSE39582. (A) The expression level of *ITLN1* mRNA was different between tumour tissues and normal tissues. (B) ROC curves were generated to verify the diagnostic performance of *ITLN1* expression differences. (C) KM survival curve of the *ITLN1* high-expression group and low-expression group. The cut-off of the group was the median expression of *ITLN1*. The significance of the prognostic value of the KM curve was tested by the log-rank test. (D-F) Analysis of the BP, CC and MF enrichment of the genes strongly associated with *ITLN1*. (G) KEGG analysis of genes strongly associated with *ITLN1*. CRC, colorectal cancer; GEO, Gene Expression Omnibus; KM, Kaplan-Meier; ROC, receiver operating characteristic; AUC, area under the curve; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

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based radiomic, pathological, and immunological data to predict OS and disease-free lung metastasis survival (DFS) in CRC patients. However, their study sample consisted of only 103 CRC patients and was limited to patients with lung metastases (from Fudan University Shanghai Cancer Center); therefore, the results were not fully representative of CRC patients (34). The advantage of our nomogram is that it was explored in a relatively large cohort and independently verified in an external database with a large sample size to ensure good generalizability and clinical applicability. It is easy to use and can be used as a fast and effective tool to personalize prognosis prediction and guide treatment for CRC patients.

This study has several limitations. Some clinicopathological factors (tumour recurrence/metastasis, obesity, chemotherapy, radiotherapy, colon polyp, circumspect margin, etc.) in the TCGA and GEO databases are incomplete or missing, so large-scale clinical trials need to be performed to fully evaluate the prognostic value of *ITLN1* in COAD.

Conclusions

In recent years, the incidence of CRC has been increasing annually. Although great progress has been made in terms of diagnosis and treatment, the survival of patients is still limited. Identifying new biomarkers with diagnostic and prognostic value is an important research direction for improving the early detection rate and prognosis of CRC. This study revealed that *ITLN1* was significantly underexpressed in CRC tissues and could be used as an effective differentiating tool for CRC and that *ITLN1* expression was significantly correlated with the survival and prognosis of CRC patients, indicating that *ITLN1* could be used as a new therapeutic target for CRC. Subsequently, a nomogram based on the risk score of *ITLN1* and clinicopathological factors was constructed. Our study explored the impact of *ITLN1* on the diagnosis and prognosis of CRC, provided a basis for further study of the MF of *ITLN1*, and provided new insights for the mechanistic exploration and treatment of CRC.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Wannan Medical College [No. (2023) 215], and informed consent was obtained from all individual participants.

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