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ORIGINAL RESEARCH

IGFBP5 is Upregulated and Associated with Poor Prognosis in Colorectal Cancer

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Purpose: This study aimed to investigate the role of IGFBP5 in colorectal cancer (CRC) and the relationship between the expression of IGFBP5 and clinicopathological parameters in CRC patients.

Patients and Methods: Immunohistochemical analysis was used to detect the expression of IGFBP5 and its correlation with clinicopathological parameters of CRC patients. Prognosis analysis, gene set enrichment analysis, and protein interaction network analysis were performed using bioinformatics analysis. The Genomics of Drug Sensitivity in Cancer (GDSC) dataset was used to analyze the correlation between the expression of IGFBP5 and drug resistance.

Results: Immunohistochemical analysis revealed that the expression of IGFBP5 was significantly higher in CRC tissues than in para-cancerous tissues ($P < 0.05$). High expression of IGFBP5 was associated with tumor differentiation and the N stage of CRC ($P < 0.05$). Moreover, high expression of IGFBP5 predicted worse overall survival and disease-free survival in CRC patients ($P < 0.05$). The expression of IGFBP5 was associated with cell–matrix adhesion, extracellular matrix binding, and collagen binding ($P < 0.05$). Furthermore, IGFBP5 was involved in the Hedgehog signaling pathway and PI3K-Akt signaling pathway ($P < 0.05$). IGF1, IGF2, SPP1, LTBP1, and FAM20C were most closely related to IGFBP5.

Conclusion: The expression of IGFBP5 is upregulated and associated with tumor differentiation, lymph node metastasis, drug resistance, and prognosis in CRC patients.

Keywords: CRC, IGFBP5, IHC, prognosis, PI3K-Akt signaling pathway

Introduction

Colorectal cancer (CRC) is one of the most malignant tumors with high morbidity and mortality worldwide and is the most common malignancy of the digestive tract.[1,2] In recent years, due to the improvement of people's standard of living, tremendous changes in the dietary habits, and increase in average life expectancy, the morbidity and mortality associated with CRC have been increasing worldwide. CRC accounts for an increasing proportion of all cancer-related deaths.[3] Epidemiological statistics have revealed that factors such as gene mutations, abnormal amplification, and changes in genetic susceptibility play key roles in the occurrence and development of CRC.[3] Research and exploration of the molecular biology of CRC oncogenes and tumor suppressor genes has improved the understanding of researchers regarding the causes, diagnosis, treatment, and risk factors of CRC. However, more relevant molecular mechanism research is needed. Therefore, it is essential to identify novel biological markers with diagnostic value for the early diagnosis of CRC and evaluation of prognosis in CRC patients.

The insulin-like growth factor-binding protein family (IGFBP) includes 7 members. These 7 members, IGFBP 1–7, play a vital role in metabolism. For example, they physically interact with IGFs (IGF1 and IGF2) and act as carriers, thereby protecting IGFs from proteolytic degradation. In addition, they interact with other proteins, such as cell surface proteins, extracellular matrix proteins, and intracellular molecules.[4] IGFBPs have been regarded as oncogene in a variety of tumors, such as IGFBP7 in breast cancer,[5] esophageal cancer[6] and hepatocellular cancer,[7] IGFBP1/2/4/5/6 in ovarian cancer.[8] IGFBP5 is a member of this family and plays an important role in cell growth, cell differentiation, apoptosis, and

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cell metabolism.[9,10] Studies have shown that IGFBP5 is related to liver steatosis and nonalcoholic steatohepatitis scores, suggesting that IGFBP5 may be involved in metabolic syndrome.[11] In addition, the absence of IGFBP5 leads to mild glucose intolerance and aggravates diet-induced obesity.[12] At present, research on IGFBP5 has been mainly focused on breast cancer. IGFBP5 acts as a key gene in tumor cell biology and can be used to predict lymph node metastasis in breast cancer patients.[13,14] In addition, IGFBP5 is involved in the occurrence and development of various tumors, such as melanoma, osteosarcoma, gastrointestinal stromal tumor, and hepatocellular carcinoma[15–18] In colon cancer, the exogenous expression of IGFBP5 activating the Wnt/β-catenin signaling transduction, promoting proliferation and inhibiting apoptosis in LoVo cells.[19] Previous studies have indicated that IGFBP5 may be involved in promoting the progression and metastasis of CRC[20–22] Nevertheless, to the best of our knowledge, no research has been conducted on the expression of IGFBP5 in CRC.

We performed an immunohistochemical analysis combined with bioinformatics analysis to study the expression of IGFBP5 in CRC and its relationship to clinical parameters, prognosis, and drug resistance in CRC patients. Our study provides a theoretical basis for the preliminary diagnosis and the molecular mechanisms underlying the occurrence and development of CRC.

Materials and Methods

Patients and Tissue Samples

CRC tissues and para-cancerous tissues were obtained from patients who received routine surgical treatment at General Surgery at the Fuyang Hospital, Anhui Medical University, from 2013 to 2018. There were 56 pathologically confirmed cases of cancer and paired normal tissues in all cases. There were 40 cases of colon cancer and 16 cases of rectal cancer. The patient age ranged from 18 to 64 years, with a median age of 56 years. In total, 36 cases pertained to male patients, and the remaining 20 cases pertained to female patients. Furthermore, 31 cases had moderately to highly differentiated adenocarcinoma, and 25 cases had poorly differentiated adenocarcinoma. None of the patients were treated with radiotherapy or chemotherapy before surgery. Immediately after surgical resection, tissues were fixed with 10% neutral formalin, dehydrated with paraffin, embedded in paraffin, and cut continuously at a thickness of 2.5 μm for immunohistochemical analysis. The study was conducted according to the principles of the Declaration of Helsinki, all the cases used in this study were approved by the Academic Committee of Anhui Medical University. Written informed consent was obtained from the study participants prior to the study commencement.

Immunohistochemical Analysis

An ultra-sensitive SP immunohistochemical method was used in this study, and the following staining steps were performed according to the reagent specifications: (1) Repair antigen: hydrogen peroxide solution (3%) was added to the section, and the sections were then incubated at 25°C for 15 min before antigen repair to remove endogenous peroxidase. A microwave oven was used to repair antigens in citrate antigen retrieval solution for 5 min, then automatically cooled at 25°C. (2) Block antigen: goat serum solution was used to block antigens for 1 h at 25°C. (3) Primary antibody incubation: rabbit anti-human polyclonal antibody IGFBP5 (HPA059827, Sigma, USA) was diluted to a concentration of 1:500 and incubated in a refrigerator at 4°C for 8 h. (4) Secondary antibody incubation: the primary antibody was washed off by PBS, then the section was incubated with horseradish peroxidase-labeled 1:200 diluted Horseradish Peroxidase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (Dako, USA) at 25°C for 1 h. (5) Chromogenic reaction: the secondary antibody was washed off by PBS, then the section was incubated with horseradish peroxidase-labeled DAB chromogenic solution (Maixin, China) for the chromogenic reaction. PBS was used instead of the primary antibody as a negative control, and the known positive slides donated by DAB reagent were regarded as positive control. The expression of IGFBP5 was scored by two blinded pathologists under a microscope. Each slice was randomly selected with four high-power fields (400×). Based on the percentage of positive cells in the field of view and the degree of positivity, the semi-quantitative integral method was used for scoring and grading. The criteria were as follows: (1) percentage of positive cells: 0 points for non-observed positive cells, 1 point for <25% positive cells, 2 points for 25–75% positive cells, 3 points for >75% positive cells and (2) degree of positivity: 0 points for no color, 1 point for

pale yellow, 2 points for claybank, and 3 points for tawny. The two scores were added together: 0–1 point was considered negative, 2–3 points was considered weakly positive, 4–5 points was considered moderately positive, and 5–6 points was considered strongly positive. Negative and weakly positive results were judged as low expression, and moderately and strongly positive results were judged as high expression.

Oncomine

The expression of IGFBP5 in cancers was assessed using the Oncomine database (https://www.oncomine.org//resource//login. html) according to the following steps: (1) gene: IGFBP5, (2) data type: all, and (3) analysis type: cancer versus normal analysis; a significant difference threshold was set at $P < 0.05$.

Gepia2

The GEPIA2 website (www.gepia2.cancer-pku.cn) is an online tool that can be used to analyze tumor tissues and normal tissues from The Cancer Genome Atlas (TCGA) and the GTEx databases. It can be used to analyze specific genes or gene sets in tumor tissues and for comparison with normal tissues, and clinical parameter analysis and survival analysis of patients can also be accessed.[23]

LinkedOmics

The LinkedOmics website (http://www.linkedomics.org/login.php) is an online tool that can be used to analyze tumor molecules and clinical levels. The LinkedOmics database contains multiomics data and clinical data for 32 cancer types from TCGA project. It is also the first multiomics database that integrates mass spectrometry-based global proteomics data, generated by the Clinical Proteomic Tumor Analysis Consortium, on selected TCGA tumor samples.[24]

Survival Analysis

The survival analysis was based on the GEPIA2 website. According to gene expression, CRC patients were divided into two groups—50% low-expression group and 50% high-expression group. The following steps were followed: (1) enter the gene name IGFBP5, (2) select the overall survival or disease-free survival. (3) select the tumor type (select both COAD and READ), and (4) visual analysis. GEPIA2 uses the Log rank test for hypothesis testing and Logrank $P < 0.05$, which was considered statistically significant.

Gene Set Enrichment Analysis

The LinkedOmics website was used to analyze the correlation between the expression of IGFBP5 and clinicopathological parameters of CRC patients. We used the Link Interpreter module for GO and KEGG analyses. The following steps were followed: (1) cancer cohort select: colorectal cancer (COADREAD), (2) search dataset selection: RNAseq, (3) search attribute select: IGFBP. (4) target dataset selection: RNAseq, (5) statistical method select: Spearman correlation analysis, (6) submitting the results of the previous step, selected GO and KEGG in GSEA for enrichment analysis. A false discovery rate (FDR) of <0.05 was used as the cutoff value for GO term and KEGG enrichment analysis.

Protein–Protein Interaction Network Construction and Module Analysis

To investigate the molecular mechanisms of IGFBP5-related genes, Cytoscape 3.7.2 software (https://cytoscape.org/) was used to search for IGFBP5 coexpressed proteins and construct the IGFBP5-related gene target network. The STRING database was used to construct the protein–protein interaction (PPI) network using the following steps: (1) single protein by name: searched for IGFBP5, (2) organism: selected "Homo sapiens" and (3) clicked "continue". The result showed the "Predicted Functional Partners" of IGFBP5. The results were intersected to obtain the gene most closely related to IGFBP5.

Methylation-Related Analysis

DNA methylation data were obtained from LinkedOmics, and the DNA methylation levels of IGFBP5 and gene expression were compared. The Methylation-related analysis was performed in LinkedOmics using the following

steps: (1) cancer cohort select: colorectal cancer (COADREAD), (2) search dataset selection: RNAseq, (3) search attribute select: IGFBP, (4) target dataset selection: methylation 450, (5) statistical method select: Spearman correlation analysis. (6) submitting the results of the previous step. Spearman correlation analysis was performed to reveal the association between methylation level and gene expression, and a false discovery rate (FDR) of <0.05 was used as the cutoff value.

Analysis of Data Derived from the GDSC

The GDSC (https://www.cancerrxgene.org/) contains expression data of IGFBP5 for 46 CRC cell lines, drug response data for anticancer compounds, and experimental data on drug sensitivity.[25] For preparation, the following files for downloaded: "cell lines details", "screened compounds", and "fitted dose response". Drug sensitivity (IC50) values were predicted using the R software v 3.5.1 (https://www.r-project.org/). Before processing, the median expression of IGFBP5 was used as a threshold to define the low expression and high expression of IGFBP5. Pearson correlation analysis was performed to detect the correlation between the expression of IGFBP5 and drug response data. $P < 0.01$ was set as the threshold. IGFBP5 gene-related drugs in CRC were screened from all compounds and visualized using the R package (seen in Availability of Data and Materials, Declarations).

Statistical Analyses

SPSS (version 23.0; SPSS Inc., Chicago, IL, USA) was used to analyze the data. The chi-square test was used to analyze the correlation between the expression of IGFBP5 and the clinicopathological parameters in CRC patients. In survival curve analysis, the Log rank test was used to compare the differences between the survival rates. Gene enrichment analysis showed that the gene set with $P < 0.05$ and FDR < 0.25 was a significantly enriched gene set. Statistical significance was set at $P < 0.05$.

Results

Expression of IGFBP5 is Upregulated in CRC Tissues

We conducted an immunohistochemical analysis to detect the protein expression of IGFBP5 in the 56 CRC tissues and para-cancerous tissues. The positive expression of IGFBP5 was mainly localized in the cytoplasm (Figure 1). We found a high expression of IGFBP5 in 78.6% (44/56) CRC tissue samples and a low expression of IGFBP5 in 21.4% (12/56) CRC tissue samples, compared to that in para-cancerous tissue samples ($P < 0.05$). We then compared the expression levels of IGFBP5 in cancer tissues and normal tissues using the Oncomine database and found that the expression levels of IGFBP5 in brain and central nervous system cancer, breast cancer, colorectal cancer, lung cancer, prostate cancer, lymphoma, and pancreatic cancer were significantly increased. For the high IGFBP5 expression in a variety of tumors, IGFBP5 may be a tumor related gene (Figure 2A). The results further demonstrated that the expression of IGFBP5 is upregulated in CRC tissues.

High Expression of IGFBP5 in CRC Was Closely Associated with Aggressive Characteristics of CRC

The 56 CRC tissues were divided into an IGFBP5 high-expression group and an IGFBP5 low-expression group according to IGFBP5 expression level. IGFBP5 immunoreactivity scores showed that the high expression of IGFBP5 was associated with tumor differentiation and the N stage ($P < 0.05$; Table 1). However, no statistically significant difference was detected between the expression of IGFBP5 and age, sex, T stage, and M stage ($P > 0.05$; Table 1).

Expression of IGFBP5 Predicts Poor Prognosis in CRC Patients

Survival analysis of 56 cases of CRC included in this study showed that the overall survival of the high IGFBP5 expression group (n = 135) was significantly shorter than that of the low IGFBP5 expression group (n = 135; $P = 0.014$; hazard ratio [HR] = 1.8; P [HR] = 0.016; Figure 2B). Similarly, the disease-free survival (DFS) of the high IGFBP5 expression group (n = 135) was significantly shorter than that of the low IGFBP5 expression group (n = 135; $P = 0.0082$; HR = 1.9; P [HR] = 0.0095; Figure 2B). We then analyzed the relationship between the expression of IGFBP5 and the

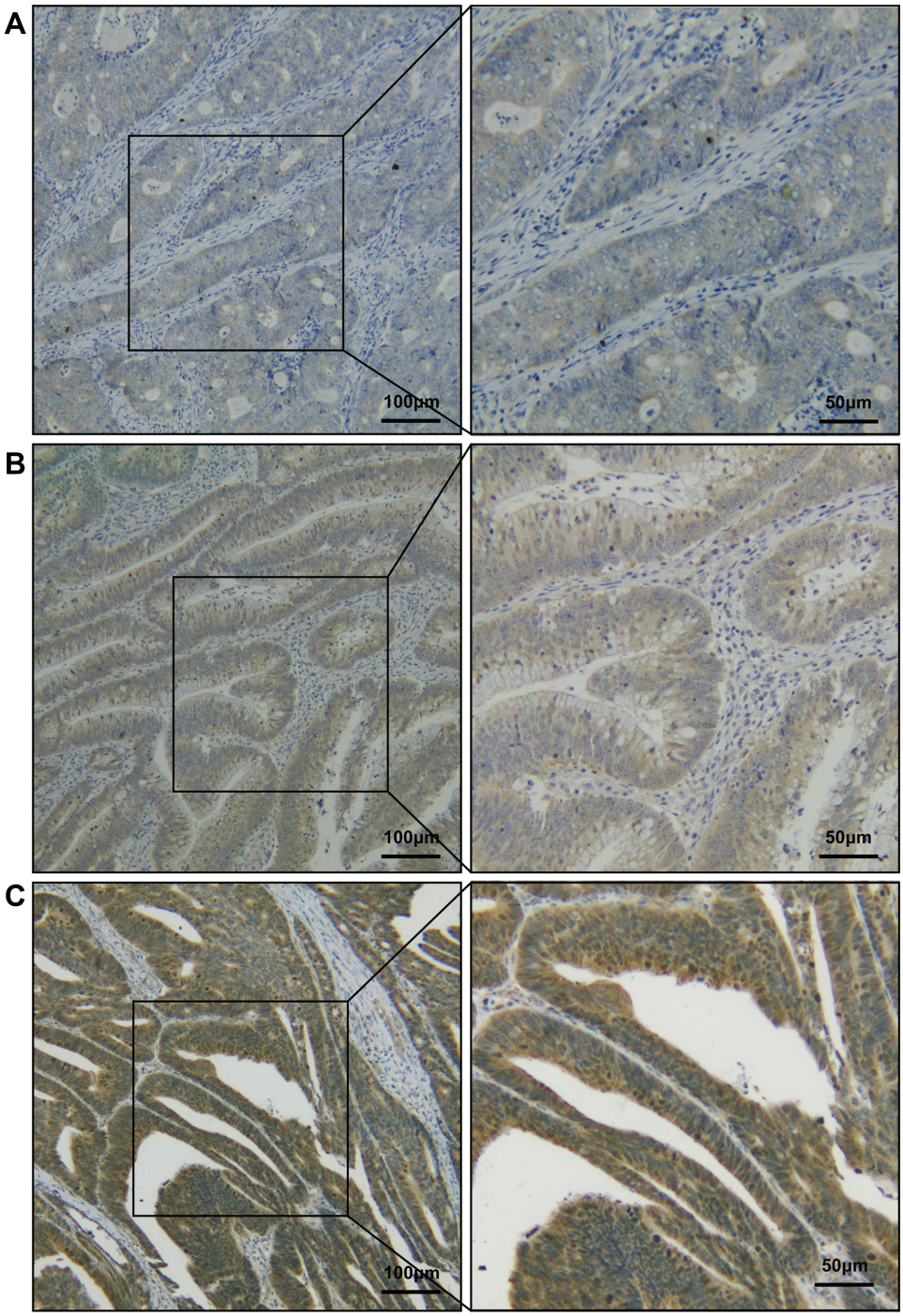

Figure 1 Representative immunohistochemical staining of IGFBP5 in tumor tissues and matched para-cancerous tissues of CRC. (**A**) Weak positivity of IGFBP5 expression in CRC tissues; (**B**) Moderate positivity of IGFBP5 expression in CRC tissues; (**C**) Strong positivity of IGFBP5 expression in CRC tissues.

prognosis of CRC patients by collecting the prognostic data of all clinical patients. Patients in our study were followed for 42–84 months, with a median follow-up of 62 months. Survival analysis using the Log-Rank method showed that patients with lower IGFBP5 expression had a longer overall survival (OS) (42.6 months, 95% confidence interval (CI): 0.1473–1.015) than patients with higher IGFBP5 expression (33.9 months, 95% CI: 0.9855–6.788) (P=0.0443, Figure 2C). Meanwhile, the DFS survival curve showed that the DFS of patients with lower IGFBP5 expression (29.5 months, 95% CI: 0.1717–1.160) than patients with higher IGFBP5 expression (24.7 months, 95% CI: 0.8617–5.825)

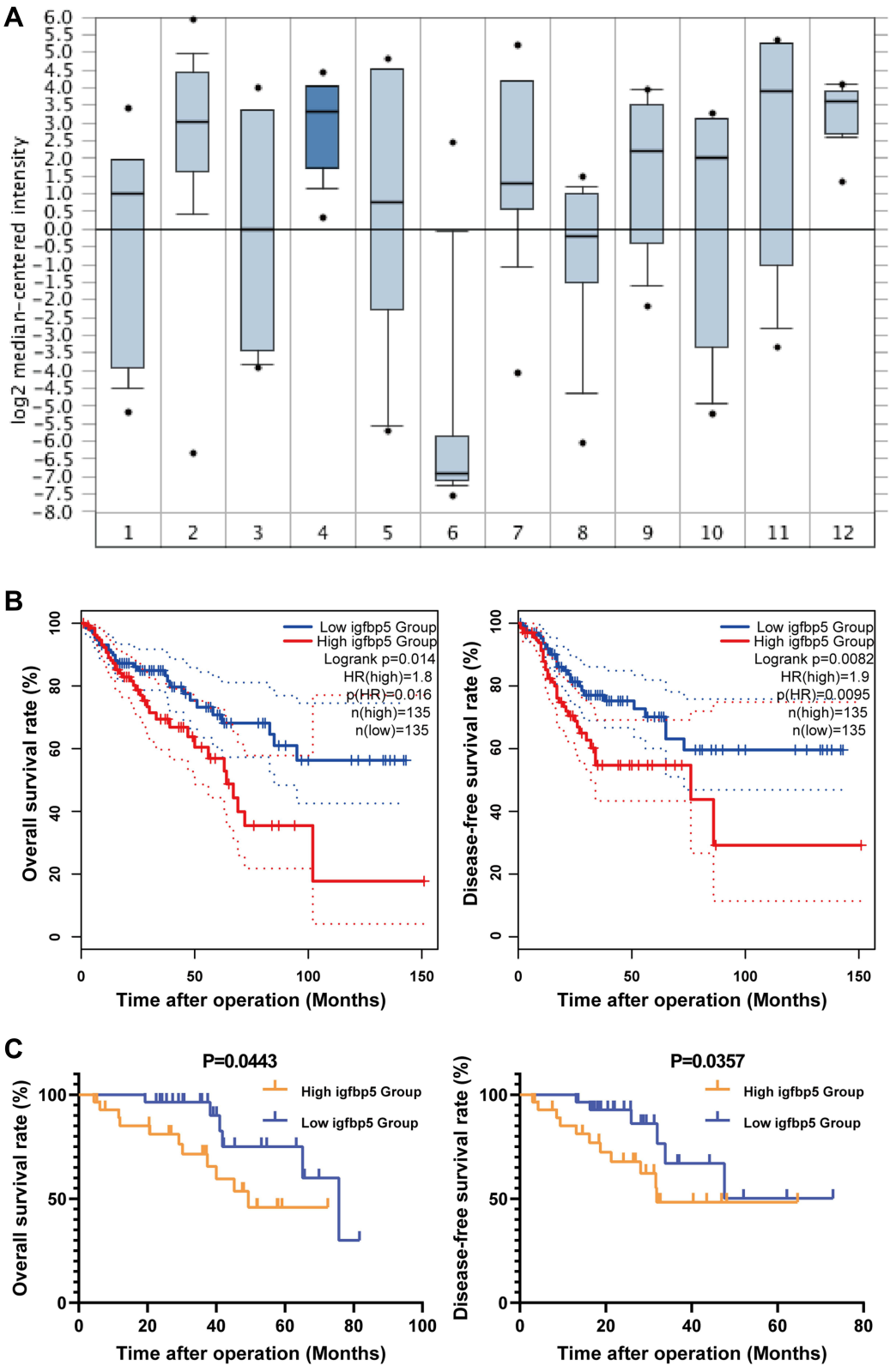

Figure 2 The expression and significance of IGFBP5 in TCGA datasets and CRC tissues. (**A**) The expression of IGFBP5 in Ramaswamy Multicancer, Oncomine. Cancer types are as follows: 1) bladder cancer, 2) brain and central nervous system cancer, 3) breast cancer, 4) colorectal cancer, 5) kidney cancer, 6) leukemia, 7) lung cancer, 8) lymphoma, 9) melanoma, 10) ovarian cancer, 11) pancreatic cancer, and 12) prostate cancer. (**B**) Correlation between relative IGFBP5 expression levels and overall survival (OS) or disease-free survival (DFS) analyzed 56 cases of TCGA sample. (**C**) Correlation between relative IGFBP5 expression levels and overall survival (OS) or disease-free survival (DFS) analyzed using 56 cases of clinical sample.

Table 1 Relationship Between Protein Expression of IGFBP5 and Clinicopathological Parameters of CRC

Features	n	IGFBP5 Expression		χ^2 value	*P* value
		Low	High		
Age (years)					
<56	28	12	16	1.143	0.285
≥56	28	16	12		
Gender					
Male	36	18	18	0.000	1
Female	20	10	10		
Differentiation					
High/middle	31	21	10	8.743	0.003*
Low	25	7	18		
T stage					
1–2	30	16	14	4.133	0.127
3	18	6	12		
4	8	6	2		
N stage					
0	36	24	12	13.333	0.001*
1	12	4	8		
2	8	0	8		
M stage					
0	48	23	25	0.146	0.705
1	8	5	3		

Note: *Values with statistical difference.

(P=0.0357, Figure 2C). Moreover, univariate analysis indicated that the IGFBP5 expression, N stage and M stage were closely correlated with the OS and DFS of patients with CRC (Table 2). Multivariate analysis showed that the IGFBP5 expression and N stage were independent risk factors for OS and DFS in postoperative patients with CRC (Table 3).

Results of Functional Gene Set Enrichment

The LinkedOmics database was used to export the top 1000 genes related to the expression level of IGFBP5 in CRC, and the molecular function and related molecular mechanism of IGFBP5 in CRC were analyzed on the DAVID website. GO analysis of cellular components showed that IGFBP5 was associated with the extracellular matrix, proteinaceous extracellular matrix, collagen trimer, and cell–cell junction. Furthermore, the results of GO analysis of biological process showed that IGFBP5 was associated with cell adhesion, extracellular matrix organization, extracellular matrix disassembly, and substrate adhesion-dependent cell spreading. The results of GO analysis of molecular function showed that IGFBP5 was related to extracellular

Table 2 Univariate Analysis of Variables Affecting the OS and DFS of Patients with CRC

Variables	OS			DFS		
	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.105	0.634–1.333	0.226	1.186	0.688–1.574	0.330
Gender	0.844	0.510–1.432	0.425	0.997	0.635–1.703	0.491
Differentiation	0.684	0.301–0.831	0.065	0.787	0.552–0.987	0.110
T stage	0.550	0.280–0.792	0.030	0.807	0.406–1.948	0.114
N stage	0.772	0.312–0.691	0.010*	0.678	0.594–1.219	0.009*
M stage	0.370	0.225–0.627	0.009*	0.362	0.144–0.565	0.001*
IGFBP5 expression	0.418	0.123–0.795	0.044*	0.606	0.315–0.870	0.036*

Note: *Values with statistical difference.

Table 3 Multivariate Analysis of Variables Affecting the OS and DFS of Patients with CRC

Variables	OS			DFS		
	HR	95% CI	P-value	HR	95% CI	P-value
N stage	0.712	0.313–1.276	0.049*	0.885	0.607–1.401	0.178
M stage	0.382	0.212–0.569	0.004*	0.573	0.134–0.809	0.007*
IGFBP5 expression	0.444	0.130–0.753	0.040*	0.652	0.299–0.834	0.038*

Note: *Values with statistical difference.

matrix structural constituents, collagen binding, extracellular matrix binding, and vascular endothelial growth factor ($P < 0.05$, Figure 3A–C). KEGG analysis indicated that Hedgehog and PI3K-Akt signaling pathways were the most significantly enriched pathways (Figure 3D). Enrichment analysis indicated that the IGFBP5 coexpressed genes were mainly involved in the regulation of the extracellular matrix, which may play an essential role in CRC progression.

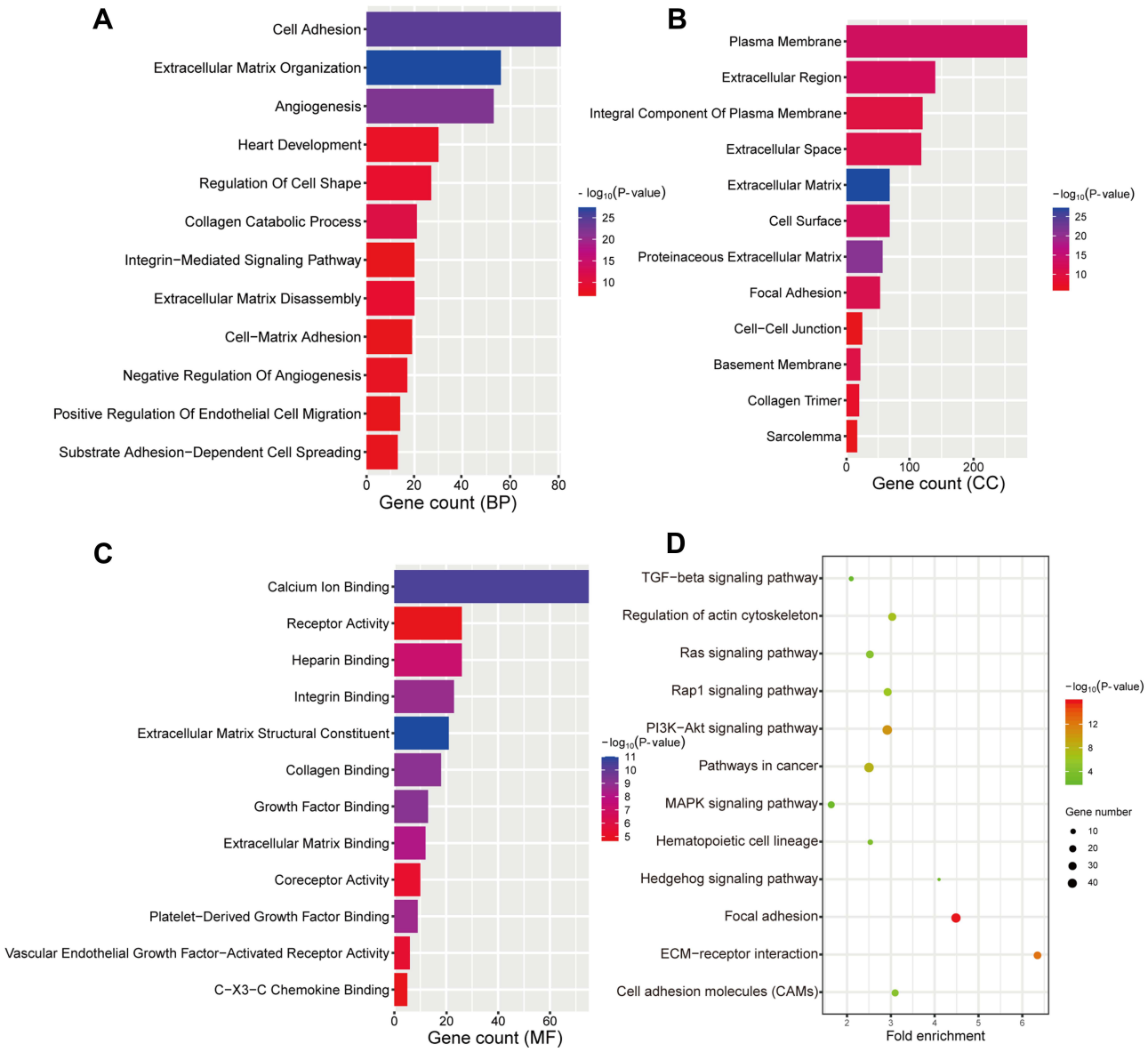

Figure 3 GO (**A–C**) and KEGG (**D**) analysis of IGFBP5 in functional gene sets of colorectal cancer.

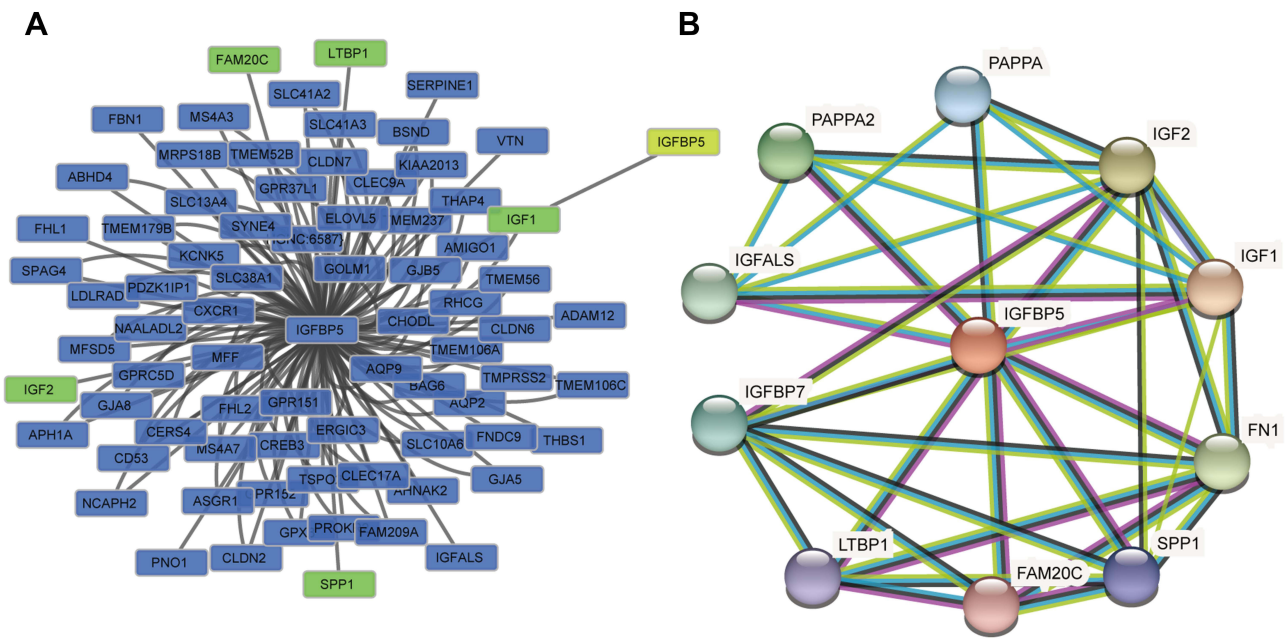

Figure 4 IGFBP5-related gene target network (**A**) and protein interaction network (**B**) in CRC analyzed using TCGA datasets.

Construction of the PPI Network and Module Analysis for IGFBP5 Coexpressed Genes

To obtain potential upstream and downstream molecules related to the enriched biological functions and related signaling pathways, we constructed an IGFBP5-related gene target network using Cytoscape 3.7.2 software (Figure 4A). We then searched for IGFBP5 coexpressed proteins using the STRING database, and constructed the IGFBP5 molecular regulatory network (Figure 4B). The results showed that the IGFBP5 coexpressed genes IGF1, IGF2, SPP1, LTBP1, FAM20C, and SSP1 were the most closely related to IGFBP5. Methylation analysis indicated that the methylation of these genes was related to the expression of IGFBP5 (Table 4).

High Expression of IGFBP5 is Associated with Increased Drug Resistance

The results of the GDSC analysis showed that GNF-2 and TGX221 were significant compounds for high IGFBP5 expression in CRC (Figure 5). The GDSC showed that TGX221, a target of PI3K beta, acted as a direct target of PI3K-Akt signaling. TGX221 conferred selective increase in drug resistance in CRC cells with high expression of IGFBP5, which makes IGFBP5 a potential individualized target for CRC patients. Our results revealed that the expression of IGFBP5 increased the drug-resistance to GNF-2 and TGX221, IGFBP5 may also play a role in CRC drug-resistance.

Table 4 The Methylation Level of IGFBP5 is Correlated with Co-Expressed Genes

Genes	Statistic	P-value	FDR
IGFBP5	−0.135	0.009	0.023
SPP1	−0.083	0.011	0.020
IGF1	−0.358	< 0.001	< 0.001
IGF2	−0.224	0.0171	0.040
FAM20C	−0.227	< 0.001	< 0.001
LTBP1	−0.119	0.025	0.056

Abbreviation: FDR, false discovery rate.

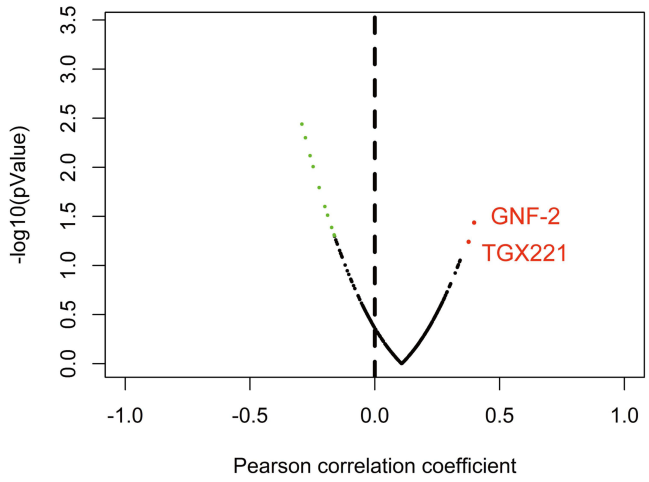

Figure 5 The GDSC dataset indicates that IGFBP5 is related to drug resistance of CRC cells; X-axis indicates correlation coefficients, and Y-axis indicates P value.

Discussion

CRC is a leading cause of cancer-related deaths worldwide.[26] Thus, identifying a reliable diagnostic marker for CRC continues to be a significant research topic. Despite improved diagnostic and treatment strategies, the overall survival of patients with CRC remains poor.[27] The occurrence and progression of CRC is a multistage process involving a variety of changes at the gene level, in which IGFBP5 may play a role in CRC. Our study aimed to identify the frequent aberrant expression of IGFBP5 in CRC and explore its prognostic significance.

IGFBP5 gene is located on the human chromosome 2q35. IGFBP5 belongs to the IGFBP family and encodes a multichannel transmembrane protein containing 272 amino acid residues with a molecular weight of 30.5 kDa.[18] In this study, we found that the expression level of IGFBP5 in CRC tissues was significantly higher than that in paired normal tissues using immunohistochemical analysis. Further analysis of clinical parameters and clinical samples showed that the expression of IGFBP5 was significantly correlated with the CRC grade and N stage ($P < 0.05$). There was no significant correlation between the expression of IGFBP5 and age, sex, T stage, and M stage ($P > 0.05$). In brief, the above-mentioned results suggested that the expression of IGFBP5 was high in CRC and its upregulation might be associated with invasion and tumor differentiation of CRC.

Previous studies have demonstrated the prognostic value of IGFBP5 in other malignancies, such as ovarian cancer and breast cancer.[28,29] We conducted survival analysis of CRC patients online using the GEPIA2 website. These results indicated that patients with high expression of IGFBP5 had shorter OS and DFS than those with low expression of IGFBP5. In addition, we concluded that IGFBP5 was an independent prognostic factor for CRC patients after surgery by using the Cox proportional hazards model.

Moreover, the high expression of IGFBP5 indicated increased drug resistance to GNF-2 and TGX221. GNF-2 is an allosteric inhibitor of Bcr-Abl which developed as a new class of anti-cancer drug to treat resistant chronic myelogenous leukemia.[30] TGX221 is a PI3Kb inhibitor targeted cancer cells with CDKN2A and PTEN mutations. TGX221 also substantially and selectively inhibited the downstream products of VHL, SETD2, and PTEN in ccRCC cells with VHL and SETD2 mutations.[31] Though GNF-2 and TGX221 were currently not used in the treatment of CRC, IGFBP5 is expected to be a new target to overcome the drug-resistance for CRC therapy.

The factors of tumor development and its complexity are in the process of malignant transformation, mostly accompanied by a decrease in cell adhesion levels and changes in the extracellular matrix state.[32] PPI network analysis identified IGF1, IGF2, SPP1, LTBP1, and FAM20C as IGFBP5 coexpressed genes, of which IGFI and IGF2 are important regulators of IGFBP5[33] and SPP1 is the upstream regulator of the PI3K-Akt signaling factor.[34] The results of a methylation analysis showed that IGFBP5 might be involved in the methylation of IGF1, IGF2, SPP1, LTBP1, FAM20C, and SSP1, thus regulating the activation of the PI3K-Akt signaling pathway.

The specific regulatory mechanism of IGFBP5 in CRC is not yet clear. The TGF-β signaling pathway is finely regulated at different levels, including the regulation of ligands, receptors, Smads, and the transcriptional level in the nucleus. Its regulation mechanisms are diverse, including protein–protein interactions, post-translational protein modification, protein degradation, protein transport and intracellular localization, and Smad-DNA binding, etc.[35] PI3K-Akt can be activated by a variety of different types of cellular stimuli and toxins and is involved in the regulation of many fundamental cellular processes, including cell growth, transcription, translation, cell proliferation, cell motility and glycogen metabolism.[36] They are both important signaling pathways in the process of tumorigenesis and development.[37,38] Status changes in DNA methylation lead to cell proliferation, apoptosis, cell adherence, cell cycle regulation, and DNA damage repair, which have been regarded as prospective targets for the development of diagnostic, prognostic, and predictive biomarkers for cancer.[39,40] In addition, promoter methylation of the PI3K-Akt signaling pathway predicts poor prognosis in CRC patients.[41,42]

The advantage of this study was that it explained the problem at the gene and protein levels. This study provides a theoretical basis for future clinical research on IGFBP5 in cancer and proposes that IGFBP5 may be used as an important target for regulating the development and prognosis of CRC, thus providing a new marker for early diagnosis and treatment of CRC.

Conclusion

In summary, we found that the expression of IGFBP5 was upregulated in CRC tissues and that its expression was correlated with tumor dedifferentiation, lymph node metastasis, drug resistance, and prognosis in CRC patients. Therefore, IGFBP5 can be used as a predictive marker for the diagnosis, treatment, and prognosis of CRC.

Data Sharing Statement

The data and R package used and analyzed during this study are available from the corresponding author (657427787@qq.com) on reasonable request.

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Disclosure

Yu Deng is the first author. The authors report no conflicts of interest in this work.

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