

Original Article

Open Access



The Inc-MAPKAPK5-AS1 promotes colon cancer-derived liver metastasis via modulating the tumor microenvironment: an *in silico* study and immunohistochemistry validation

Xiangzhi Hu^{1,2}, Dedong Wang¹, Jinbin Chen³, Ke Tang⁴, Qiqi Yan⁵, Di Wu^{1,4}

¹School of Public Health, Institute of Public Health, Guangzhou Medical University & Guangzhou Center for Disease Control and Prevention, Guangzhou Medical University, Guangzhou 510440, Guangdong, China.

²Infectious Disease Control Department, Yidu Center for Disease Control and Prevention, Yidu 443300, Hubei, China.

³Guangzhou Key Laboratory for Clinical Rapid Diagnosis and Early Warning of Infectious Diseases, KingMed School of Laboratory Medicine, Guangzhou Medical University, Guangzhou 510180, Guangdong, China.

⁴School of Public Health, Guangdong Medical University, Dongguan 523808, Guangdong, China.

⁵Department of Public Health and Preventive Medicine, School of Medicine, Jinan University, Guangzhou 510632, Guangdong, China.

Correspondence to: Dr. Di Wu, School of Public Health, Institute of Public Health, Guangzhou Medical University & Guangzhou Center for Disease Control and Prevention, Guangzhou Medical University, Xinzao Town, Panyu District, Guangzhou 510440, Guangdong, China. E-mail: gzcdc_wud@gz.gov.cn

How to cite this article: Hu X, Wang D, Chen J, Tang K, Yan Q, Wu D. The Inc-MAPKAPK5-AS1 promotes colon cancer-derived liver metastasis via modulating the tumor microenvironment: an *in silico* study and immunohistochemistry validation. *J Cancer Metastasis Treat*. 2025;11:15. <https://dx.doi.org/10.20517/2394-4722.2025.26>

Received: 27 Feb 2025 **First Decision:** 30 May 2025 **Revised:** 20 Jun 2025 **Accepted:** 9 Jul 2025 **Published:** 17 Jul 2025

Academic Editors: Xiaofang Che, Ciro Isidoro **Copy Editor:** Fangling Lan **Production Editor:** Fangling Lan

Abstract

Background: This study aimed to investigate the expression of *Inc-MAPKAPK5-AS1* in colon cancer and its clinical implications for prognosis, liver metastasis, and tumor microenvironment (TME) regulation.

Methods: Through integrative analysis of public databases (TCGA, GEO, cBioPortal, David, BioGRID, etc.), we systematically evaluated the roles of *Inc-MAPKAPK5-AS1* and *MAPKAPK5* in colon cancer development, liver metastasis, immune microenvironment modulation, and associated biological pathways. Immune infiltration patterns were assessed using ESTIMATE, CIBERSORT, and xCell algorithms. *MAPKAPK5* protein expression was further validated by immunohistochemistry.

Results: *Inc-MAPKAPK5-AS1* was upregulated in colon cancer tissues and correlated with poor clinical outcomes.



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Immunohistochemistry confirmed that *MAPKAPK5* protein was strongly or moderately expressed in colon cancer tissues. Furthermore, compared with the colon cancer tissues *in situ*, patients with liver metastasis also showed elevated expression of *lnc-MAPKAPK5-AS1*. Functional enrichment analysis revealed that differentially expressed genes (DEGs) were primarily involved in amino acid and lipid metabolism pathways. In addition, immune infiltration analysis indicated that *lnc-MAPKAPK5-AS1* expression was associated with multiple immune checkpoint inhibitors. The group with high gene expression showed increased infiltration abundance of regulatory T cells, gamma-delta T cells, and CD8⁺ naive T cells.

Conclusions: *lnc-MAPKAPK5-AS1* may serve as a potential oncogenic biomarker in colon cancer. Our findings indicate that its upregulation could contribute to tumor progression and liver metastasis, possibly by remodeling the TME via immune cell modulation, including potential alterations in the proliferation, differentiation, and functional states of key lymphocyte subsets.

Keywords: Colon cancer, liver metastasis, *lnc-MAPKAPK5-AS1*, *MAPKAPK5*, prognosis

INTRODUCTION

Colorectal cancer is a highly prevalent and deadly malignant tumor worldwide. According to the International Agency for Research on Cancer (IARC), it ranks third in incidence among all cancers^[1]. It is the most common gastrointestinal malignancy in China, with colon cancer accounting for ~40% of cases^[2]. Furthermore, the incidence and mortality rates of colorectal cancer have been increasing over the past decade. Liver metastasis is the most common site of colorectal cancer metastasis and the leading cause of death. Current therapeutic interventions, including systemic therapy and liver resection, have been demonstrated to enhance the five-year survival rate of patients, but only ~25% of patients exhibit indications for surgical resection^[3]. A survey indicates that 83% of colorectal cancer patients in China are already in the middle or late stages at the time of diagnosis, and 44% of those patients have developed distant metastases^[4], indicating a significant challenge in effectively managing the disease. The liver, lungs, bones, and lymph nodes are frequently affected by metastasis in all malignancies. However, from another perspective, when evaluated in comparison with other malignant neoplasms, the prognosis of liver metastases from colorectal cancer is comparatively favorable. Recent advances in clinical research have enabled the widespread use of molecular biomarkers for the early diagnosis and prognosis assessment of colon cancer.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs whose transcripts are more than 200 nucleotides in length. lncRNAs are involved in the process of regulation of gene expression in organisms, mainly through interactions with DNA, RNA or proteins. Antisense lncRNA refers to a class of RNA molecules that are transcribed by antisense chains of protein-coding genes and have partial sequences that overlap with their mRNA^[5]. It is imperative to note that antisense lncRNAs frequently demonstrate a correlation with the expression of their sense genes. These molecules hold considerable potential to serve as novel biomarkers and therapeutic targets for neoplasms, as evidenced in recent studies^[6]. For example, *lnc-SLCO4A1-AS1* acted as an oncogene in colon cancer and affected tumor stemness properties by positively regulating *SLCO4A1*^[7]. Chen *et al.* confirmed that *lnc-GAS6-AS1* promoted *TRIM14*-mediated cell proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) in colorectal cancer through a ceRNA network and in a FUS-dependent manner^[8].

lnc-MAPKAPK5-AS1 (hereinafter *MK5-AS1*) is a natural antisense transcript originating from the opposite strand of the *MAPKAPK5* (hereinafter *MK5*) gene locus. This cis-regulation configuration suggests potential functional interplay. It has been found to be dysregulated in a variety of diseases. A body of

research has previously indicated the significance of the function of *MK5-AS1* in the development and progression of colon^[9,10]. However, comparatively limited research has been conducted on its role in the metastasis of colorectal cancer to the liver primary colon cancer tissues. The utilization of bioinformatics facilitates the acquisition of prognostic indicators through the efficient mining and analysis of data, drawing upon prior research findings. At present, liver metastasis is the predominant cause of mortality from colon cancer, so it is necessary to conduct targeted research. To elucidate the clinical and prognostic relevance of *MK5-AS1* in colon cancer progression and metastasis, we performed an integrative bioinformatics analysis of multiple public databases and validated its expression patterns via immunohistochemistry. Our findings further revealed a critical role of *MK5-AS1* in remodeling the TME. Collectively, these results suggest that upregulation of *MK5-AS1* may facilitate colon cancer liver metastasis through immune-mediated mechanisms and inflammatory pathway activation.

MATERIAL AND METHODS

Data acquisition and processing

A total of 466 colon cancer samples were included in our research, including 427 colon cancer tissues and 39 normal tissues. The inclusion criteria for the study are as follows: (i) “TCGA-COAD” or “TCGA-READ” project; (II) Patients have complete clinical data (including age, gender, tumor size, lymph node status, distant metastasis, and treatment history, etc.). Exclusion criteria: (i) Non-colorectal cancer patients; (II) Serious lack of clinical data; (III) Patients who have received special treatment or suffer from other serious diseases. All transcriptomic sequencing data and corresponding clinical information were downloaded from the TCGA database (<https://portal.gdc.cancer.gov/v1>, accessed on 15 March 2023)^[11].

Lnc-MAPKAPK5-AS1 expression was markedly increased in liver metastases relative to primary colon cancer tissues

To investigate the role of the target gene in promoting liver metastasis in colon cancer, the GSE41258 dataset was obtained from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>, accessed on 17 March 2023)^[12]. Additionally, the transcriptome data from two additional microarray datasets, GSE49355 and GSE18549, were obtained on 18 March 2023, which both included pertinent information concerning primary colon cancer tissues and colon cancer liver metastases. According to the median expression levels of *MK5-AS1* and *MK5*, the patients were divided into high and low expression groups. Additionally, the *MK5-AS1* expression was extracted in different tissue types. Subsequently, we conducted a differential analysis of three datasets, comparing liver metastasis tissues and primary cancer tissues to identify a list of up- and downregulated genes. We then screened the intersection for additional GO and KEGG analysis, with the objective of gaining insight into the potential biological role of *MK5-AS1* in colon cancer liver metastasis.

Batch effect correction and validation

Raw expression matrices were log2-transformed, and low-expressed genes (mean expression < 1) were filtered to enhance data quality. Common genes across all datasets were identified and merged into a combined expression matrix. To account for technical variability between datasets, we applied the ComBat algorithm (sva package), which utilizes parametric empirical Bayes frameworks to adjust for batch effects while preserving biological signals. The batch variable was defined based on the original GEO accession numbers. To evaluate the effectiveness of batch correction, we performed Principal Component Analysis (PCA) before and after adjustment and generated sample correlation heatmaps (pheatmap package) to assess inter-sample relationships post-normalization.

Immunohistochemistry for evaluation of MAPKAPK5 protein expression

To further verify the expression pattern and prognostic significance of *MK5* at the protein level, a commercial colon cancer tissue chip (lot NO. HColA180su13; Shanghai, China) was procured. The microarray chip comprises 90 paired samples of colon adenocarcinoma tissues and their matched adjacent normal tissues collected from surgical patients between January and October 2009. These patients were subsequently followed up for an average of 5.7-6.5 years, during which their survival, recurrence, and metastasis statuses were meticulously documented. The IHC experiment was also conducted by the Shanghai Outdo Biotech Co., Ltd. The protocol was approved by the ethics committee of the aforementioned company (The approval code of the ethics committee is SHYJS-CP-1901001).

Construction of PPI network

BioGRID (<https://thebiogrid.org>)^[13] is a publicly accessible database that archives and disseminates genetic and protein interaction data from model organisms and humans. Furthermore, it encompasses post-translational modifications of proteins and interactions with bioactive small molecules, derived from extant literature, including seminal low-throughput studies and substantial high-throughput datasets. We retrieved *MK5* interacting proteins and utilized the PPI network to visualize the data. For each protein, detailed evidence is available online.

Functional enrichment analysis

The David database (<https://david.ncifcrf.gov>, accessed on 2 April 2023)^[14] is a valuable resource for obtaining comprehensive biological function annotation information for large-scale gene or protein lists. To investigate the potential biological processes associated with *MK5-AS1*, the top 200 genes that are closely linked to *MK5-AS1* expression were retrieved from the GEPIA database (<http://gepia2.cancer-pku.cn/#index>, accessed on 2 April 2023)^[15]. These genes were subsequently imported into the David database for GO and KEGG analysis.

Gene mutation analysis

The cBioPortal database (<https://www.cbioportal.org/>)^[16] is a powerful TCGA reanalysis platform that provides a user-friendly visual interface to display gene mutation profiles, changes in DNA copy number, DNA methylation status, and protein expression (accessed on 4 April 2023). Furthermore, somatic mutation data for colon adenocarcinoma patients were obtained from TCGA-COAD. Patients were stratified into high- and low-expression groups based on median expression levels of both *MK5-AS1* and *MK5*. Additionally, the patients' age annotations were added. Finally, we investigated the mutation disparities among the different groups and waterfall plots in Sangerbox (<http://vip.sangerbox.com/home.html>, accessed on 4 April 2023)^[17] online analysis software were used to visualize the top 20 genes exhibiting the highest mutation frequencies.

Immune infiltration analysis

To characterize the TME of colon cancer patients, we employed the “ESTIMATE” R package to compute stromal, immune, and combined ESTIMATE scores for each patient. The ImmuneScore and StromalScore quantify the relative abundance of immune and stromal components within the TME, respectively, while the ESTIMATE Score represents their cumulative measure.

For immune cell profiling, we performed deconvolution analysis of the gene expression matrix to estimate the infiltration levels of 22 distinct immune cell subtypes in each sample. Additionally, we applied xCell^[18], a single-sample gene set enrichment analysis (ssGSEA) algorithm, to determine the relative abundance of 64 cell types spanning five major categories: adaptive immune cells, innate immune cells, hematopoietic progenitor cells, epithelial cells, and extracellular matrix components.

Statistical analysis

Statistical analysis and result visualization were performed using R-4.2.1 and GraphPad Prism 8.0. The differences between the groups were compared using two-sided Student's *t* test or Mann-Whitney *U* test. A Chi-square test was applied to assess the association between gene expression and clinicopathological features. Survival analysis was implemented using the log-rank test to evaluate the difference in prognosis among different groups. To analyze the predictive power of gene expression on the prognosis of patients with colon cancer, ROC curves were developed and visualized by the “pROC” package. To minimize the impact of potential confounding factors, stratified prognostic analyses were further carried out. Univariate and multivariate Cox regressions were employed to calculate hazard ratios (*HR*) and 95% confidence intervals (*CI*), thereby identifying independent risk factors for colon cancer. *HR* values were used to assess the effect of different prognostic factors on cancer progression; if $HR > 1$, it means that the prognostic factors increase the risk of death of the patient, and vice versa, it decreases the risk of death.

In the analysis of IHC validation, a curve plot between statistical power and sample size was created using the “Tests for Two Survival Curves Using Cox's Proportional Hazards Model” module of PASS software. In our study, the probabilities of an event were set as $P_{ev1} = 0.25$ for the MK5 low expression group and $P_{ev2} = 0.82$ for the MK5 high expression group, with a *HR* of 3.28 (P_{ev2}/P_{ev1}). The findings indicated that including 90 samples in this study effectively satisfies the criteria for $HR > 3$, with statistical efficiency reaching ~90%. $P < 0.05$ was considered statistically significant.

RESULTS

Gene expression analysis

By downloading the transcriptome data from public databases, we investigated the expression patterns of *MK5-AS1* and *MK5* in colon cancer patients and normal tissues. As indicated by the results of the TCGA, the expression levels of the two aforementioned genes were found to be elevated in colon cancer tissues (Figure 1A, $P < 0.001$). The results were then verified using the GSE41258 dataset and the online prediction tool GEPIA 2.0 (Figure 1B and C; both $P < 0.05$). Furthermore, significant positive correlations were observed between the two genes in TCGA-COAD and GEO datasets ($R = 0.33$, $P = 1.7 \times 10^{-7}$; $R = 0.45$, $P = 7.7 \times 10^{-21}$; Figure 1D and E).

Batch effect assessment and correction validation

The pre-correction PCA plot [Supplementary Figure 1A] revealed clear separation of samples by their dataset origin (GSE18549, GSE41258, and GSE49355) along principal components, with distinct clustering patterns indicating substantial technical variation between platforms. After ComBat adjustment [Supplementary Figure 1B], samples showed markedly improved mixing across batches, with overlapping 95% confidence ellipses demonstrating successful reduction of non-biological variation. The first two principal components post-correction primarily reflected biological variance rather than technical artifacts. The post-correction heatmap [Supplementary Figure 1C] displayed a relatively uniform correlation pattern across all samples, without obvious block structures corresponding to the original datasets.

Correlation between gene expression and clinicopathologic features

Using the online website UALCAN (<https://ualcan.path.uab.edu/>, accessed on 5 April 2023)^[19], we employed the expression levels of *MK5-AS1* and *MK5* in colon cancer patients with varying clinical stages. As illustrated by the box plots, elevated levels of *MK5-AS1* and *MK5* were found to be associated with more advanced clinical stages [Figure 1F and G]. As shown in Table 1, cases in which the expression level of *MK5-AS1* was higher than the average of normal tissues were considered the gene overexpressed group, and vice versa, the low expression group. The *MK5-AS1* high expression group showed a higher proportion of females ($P = 0.047$), Caucasians ($P = 0.045$), and patients with synchronous colon cancer ($P = 0.037$).

Table 1. Association between *lnc-MAPKAPK5-AS1* expression and clinicopathological parameters in TCGA-COAD samples

Characteristics	n	<i>lnc-MAPKAPK5-AS1</i> expression		χ^2	P-value
		High (n = 364)	Low (n = 63)		
Sex				3.938	0.047
Male	200	164(82.0)	36(18.0)		
Female	223	198(88.8)	25(11.2)		
Age				1.868	0.172
Age ≤ 60	125	102(81.6)	23(18.4)		
Age > 60	302	262(86.8)	40(13.2)		
Race				6.218	0.045
Black	58	53(91.4)	5(8.6)		
White	206	165(80.1)	41(19.9)		
Asian	10	10(100.0)	0(0)		
Missing	153	136(88.9)	17(11.1)		
Histological type				0.015	0.904
Colon adenocarcinoma	378	323(85.4)	55(14.6)		
Colon mucinous adenocarcinoma	46	39(84.8)	7(15.2)		
Missing	3	2(66.7)	1(33.3)		
Clinical stage				5.121	0.024
I, II	258	213(82.6)	45(17.4)		
III, IV	159	144(90.6)	15(9.4)		
Missing	10	6(60.0)	4(40.0)		
T				0.304	0.581
T1, T2	84	70(83.3)	14(16.7)		
T3, T4	343	294(85.7)	49(14.3)		
N				3.963	0.047
N0	243	201(82.7)	42(17.3)		
N1	166	149(89.8)	17(10.2)		
Missing	18	14(77.8)	4(22.2)		
M				2.455	0.117
M0	305	257(84.3)	48(15.7)		
M1	62	57(91.9)	5(8.1)		
Missing	60	50(83.3)	10(16.7)		
BMI				3.880	0.049
18.5-23.9	55	41(74.5)	14(25.5)		
Abnormal value	171	147(86.0)	24(14.0)		
Missing	201	176(87.6)	25(12.4)		
Residual tumor				2.914	0.088
R0	310	259(83.5)	51(16.5)		
R1, R2	26	25(96.2)	1(3.8)		
Missing	90	80(88.9)	11(11.1)		
Anatomic neoplasm subdivision				9.294	0.026
Ascending colon	1	0(0)	1(100.0)		
Cecum	105	84(80.0)	21(20.0)		
Sigmoid colon	141	124(87.9)	17(12.1)		
Others	154	134(87)	20(13.0)		
Missing	26	22(84.6)	4(15.4)		
Lymph node examined count				0.277	0.599
≤ 10	32	26(81.2)	6(18.8)		

> 10	374	317(84.8)	57(15.2)		
Missing	21	21(100.0)	0(0)		
Venous invasion				3.575	0.059
Yes	284	235(82.7)	49(17.3)		
No	89	81(91.0)	8(9.0)		
Missing	54	48(88.9)	6(11.1)		
Lymphatic invasion				4.843	0.028
Yes	240	191(81.3)	49(17.3)		
No	145	137(94.5)	8(5.5)		
Missing	39	36(92.3)	3(7.7)		
Synchronous colon cancer present				4.339	0.037
Yes	362	304(84.0)	58(16.0)		
No	23	23(100.0)	0(0)		
Missing	42	37(88.1)	5(11.9)		
History of colon polyps				0.824	0.364
Yes	235	201(85.5)	34(14.5)		
No	127	104(81.9)	23(18.1)		
Missing	65	59(90.8)	6(9.2)		
Colon polyps present				0.007	0.931
Yes	136	113(83.1)	23(16.9)		
No	79	66(83.5)	13(16.5)		
Missing	212	185(87.3)	27(12.7)		
Loss of mismatch repair protein expression by IHC				0.666	0.415
Yes	55	45(81.8)	10(18.2)		
No	266	229(86.1)	37(13.9)		
Missing	106	90(84.9)	16(15.1)		
Number of first-degree relatives with cancer diagnosis				0.233	0.629
≤ 1	358	306(85.5)	52(14.5)		
> 1	10	8(80.0)	2(20.0)		
Missing	59	50(84.7)	9(15.3)		

P-value: Statistically significant results (in bold); TNM: tumor-node-metastasis; BMI: body mass index.

compared to the low expression group. At the same time, the higher gene expression was associated with higher levels of BMI ($P = 0.049$), N stage ($P = 0.047$), and lymphatic invasion ($P = 0.028$), which also means that these patients have a higher risk of developing stage III or stage IV ($P = 0.024$). In addition, *MK5-AS1* expression varied across anatomic neoplasm subtypes ($P = 0.026$). For its encoded protein *MK5*, as shown in Table 2, the high expression group included a higher proportion of male patients ($P = 0.023$), cases classified as adenocarcinoma ($P = 0.011$), and patients with higher N stage ($P = 0.039$). In addition, the composition of anatomical tumor subdivisions was also different in the high and low expression groups ($P = 0.001$).

The effects of overexpressed *Inc-MAPKAPK5-AS1* and *MAPKAPK5* on the prognostic significance of colon cancer patients

We systematically examined the association between transcriptional profiles and clinical outcomes in colon cancer patients. As shown in Figure 1H, it was found that the median survival time of patients with upregulated *MK5-AS1* was 1,754 days, which was significantly shorter than that of patients with low gene expression (3,923 days, $P_{\log \text{rank}} = 0.048$). The results of *MK5* exhibited the same trend; patients with low gene expression had better survival outcome ($P_{\log \text{rank}} = 1.1 \times 10^{-6}$, Figure 1I). The “pROC” R package was used to draw time-dependent ROC curves based on the patients’ follow-up time and the expression levels of the two genes to obtain different AUC values. The results showed that *MK5-AS1* had the highest relative AUC of

Table 2. Relationship between *MAPKAPK5* expression and clinicopathological parameters of COAD samples in TCGA

Characteristics	n	MAPKAPK5 expression		χ^2	P-value
		High (n = 375)	Low (n = 52)		
Sex				5.139	0.023
Male	227	207(91.2)	20(8.8)		
Female	200	168(84.0)	32(16.0)		
Age				0.334	0.563
Age ≤ 60	125	108(86.4)	17(13.6)		
Age > 60	302	267(88.4)	35(11.6)		
Race				1.179	0.555
Asian	10	10(100.0)	0(0)		
Black	58	54(93.1)	4(6.9)		
White	201	183(91.0)	18(9.0)		
Missing	158	128(81.0)	30(19.0)		
Histological type				6.507	0.011
Colon adenocarcinoma	378	337(89.2)	41(10.8)		
Colon mucinous adenocarcinoma	46	35(76.1)	11(23.9)		
Missing	3	3(100.0)	0(0)		
Clinical stage				0.064	0.801
I, II	258	225(87.2)	33(12.8)		
III, IV	159	140(88.1)	19(11.9)		
Missing	10	10(100.0)	0(0)		
T				2.479	0.115
T1, T2	84	78(92.9)	6(7.1)		
T3, T4	343	297(86.6)	46(13.4)		
N				4.244	0.039
N0, N1	337	301(89.3)	36(10.7)		
N2	72	58(80.6)	14(19.4)		
Missing	18	16(88.9)	2(11.1)		
M				0.105	0.746
M0	305	266(87.2)	39(12.8)		
M1	62	55(88.7)	7(11.3)		
Missing	60	54(90.0)	6(10.0)		
BMI				1.039	0.308
18.5~23.9	55	52(94.5)	3(5.5)		
Abnormal value	171	154(90.1)	17(9.9)		
Missing	201	169(84.1)	32(15.9)		
Residual tumor				0.928	0.335
R0, R1	320	265(85.5)	45(14.5)		
R2	26	24(92.3)	2(7.7)		
Missing	89	86(96.7)	3(3.3)		
Anatomic neoplasm subdivision				17.657	0.001
Cecum	105	87(82.9)	18(17.1)		
Ascending colon	1	1(100.0)	0(0)		
Sigmoid colon	141	129(98.5)	12(1.5)		
Others	154	134(87.0)	20(13.0)		
Missing	26	24(92.3)	2(7.7)		
Lymph node examined count				1.338	0.247
≤ 10	32	30(93.8)	2(6.2)		

> 10	374	324(86.6)	50(13.4)		
Missing	21	21(100.0)	0(0)		
Venous invasion				0.010	0.922
Yes	89	78(87.6)	11(12.4)		
No	284	250(88.0)	34(12.0)		
Missing	54	47(87.0)	7(13.0)		
Lymphatic invasion				2.140	0.143
Yes	235	210(89.4)	25(10.6)		
No	153	129(84.3)	24(15.7)		
Missing	39	36(92.3)	3(7.7)		
Synchronous colon cancer present				3.735	0.053
Yes	23	23(100.0)	0(0)		
No	362	311(85.9)	51(14.1)		
Missing	42	41(97.6)	1(2.4)		
History of colon polyps				2.614	0.106
Yes	127	104(81.9)	23(18.1)		
No	235	207(88.1)	28(11.9)		
Missing	65	64(98.5)	1(1.5)		
Colon polyps present				2.660	0.103
Yes	79	75(94.9)	4(5.1)		
No	136	120(88.2)	16(11.8)		
Missing	212	180(84.9)	32(15.1)		
Loss of mismatch repair protein expression by IHC				0.076	0.783
Yes	55	47(85.5)	8(14.5)		
No	266	231(86.8)	35(13.2)		
Missing	106	97(91.5)	9(8.5)		
Number of first-degree relatives with cancer diagnosis				1.579	0.209
≤ 1	358	309(86.3)	49(13.7)		
> 1	10	10(100.0)	0(0)		
Missing	59	56(94.9)	3(5.1)		

P-value: Statistically significant results (in bold).

0.72 for 3-year and 5-year survival rates, while the 5-year survival rate of *MK5* had the highest AUC of 0.73 [Figure 1J] and K].

In addition, we compared the survival outcomes of colon cancer patients grouped according to several key clinical characteristics. Finally, four clinicopathologic parameters were identified as statistically significant. As shown in Figure 2A-D, those classified as colon mucinous adenocarcinoma, lymphatic invasion, venous invasion, and colon without loss of mismatch repair protein expression had a worse prognosis.

Stratified analysis of the relationship between *Inc-MAPKAPK5-AS1* expression and prognosis of colon cancer patients

To control for potential confounding factors, we also performed a stratified analysis of *MK5-AS1* expression and prognosis of colon cancer patients. The results showed that in the two variable groups of No and Mo stages, *MK5-AS1* high expression vs. low expression has a significant impact on the survival of colon cancer patients ($HR_{No} = 1.56$, 95%CI = 1.32-3.51; $HR_{Mo} = 1.19$, 95%CI = 1.38-3.69), and the adjusted *HR* is detailed in Table 3. However, in our clinical samples, the results of *MK5* did not show statistically significant clinical variables [Table 4].

Table 3. Stratified analysis of the relationship between *Inc-MAPKAPK5-AS1* expression and prognosis of colon cancer

Variables	MAPKAPK5-AS1 low		MAPKAPK5-AS1 high		Adjusted HR (high vs. low)
	Cases (N)	Deaths (%)	Cases (N)	Deaths (%)	
Sex					
Male	36	3(8.30)	200	27(13.5)	0.96(0.28-3.24)
Female	27	2(7.41)	164	21(12.8)	1.75(0.54-5.70)
Age					
Age ≤ 60	23	0(0)	102	10(9.80)	3.73(0.81-17.04)
Age > 60	40	5(12.5)	262	38(14.5)	1.81(0.29-2.23)
Race					
Asian	0	0(0)	10	0(0)	-
White	36	5(13.9)	165	28(16.9)	1.09(0.41-2.9)
Others	5	0(0)	53	9(17.0)	3.93(0.62-24.85)
Missing	22	0(0)	136	11(8.09)	-
Histological type					
Colon adenocarcinoma	56	4(7.14)	323	40(12.4)	1.90(0.76-4.74)
Colon mucinous adenocarcinoma	7	1(24.3)	39	7(17.9)	1.67(0.29-9.67)
Missing	0	0(0)	2	1(50.0)	-
Clinical stage					
I, II	45	3(6.67)	213	19(8.92)	1.25(0.40-3.89)
III, IV	15	2(13.3)	144	28(19.4)	1.34(0.38-4.79)
Missing	3	0(0)	7	1(14.3)	-
T					
T1, T2	14	1(7.14)	70	6(8.57)	1.19(0.12-11.56)
T3, T4	49	4(8.16)	294	42(14.3)	1.57(0.66-3.73)
N					
N0	42	3(7.14)	201	20(9.95)	1.56(1.32-3.51)
N1, N2	17	2(11.76)	149	25(16.8)	1.50(0.44-5.12)
Missing	4	0(0)	14	3(21.4)	-
M					
M0	48	3(6.25)	257	25(9.73)	1.19(1.38-3.69)
M1	5	1(20.0)	57	16(28.1)	2.96(0.79-10.97)
Missing	10	1(10.0)	50	7(14.0)	-
BMI					
18.5-23.9	14	1(7.14)	41	5(12.2)	1.50(0.27-8.20)
Abnormal value	24	2(8.33)	147	16(10.9)	1.66(0.37-7.55)
Missing	25	2(8.00)	176	27(15.3)	-
Residual tumor					
RO, R1	51	2(3.92)	261	17(6.51)	1.05(0.24-4.58)
R2	1	0(0)	23	4(17.4)	-
Missing	11	3(27.3)	80	27(33.8)	-
Anatomic neoplasm subdivision					
Cecum	15	0(0)	90	17(18.9)	3.09(0.61-15.68)
Ascending colon	14	2(14.3)	64	8(12.5)	1.38(0.24-7.83)
Sigmoid colon	18	1(5.56)	123	11(8.94)	2.44(0.55-10.80)
Others	12	0(0)	73	8(10.9)	3.03(0.23-40.36)
Missing	4	2(50.0)	14	4(28.6)	-
Lymph node examined count					
≤ 10	6	1(16.7)	26	7(26.9)	3.60(0.10-133.7)
> 10	57	4(7.02)	317	40(12.6)	1.68(0.72-3.94)

Missing	0	0(0)	21	1(4.76)	-
Venous invasion					
Yes	8	2(25.0)	81	18(22.2)	1.10(0.24-5.05)
No	49	2(4.10)	235	23(9.79)	2.15(0.73-6.39)
Missing	6	1(16.7)	48	7(14.6)	-
Lymphatic invasion					
Yes	16	2(12.5)	137	22(16.1)	1.02(0.23-4.42)
No	44	2(4.55)	191	20(10.5)	1.87(0.59-5.93)
Missing	3	1(33.3)	36	6(16.7)	-
Synchronous colon cancer present					
Yes	0	0(0)	23	1(4.35)	-
No	58	4(6.90)	304	35(11.5)	1.53(0.63-3.72)
Missing	5	1(20.0)	37	12(32.4)	-
History of colon polyps					
Yes	23	2(8.70)	104	6(5.77)	1.25(0.22-7.02)
No	34	2(5.88)	201	26(12.9)	1.87(0.60-5.79)
Missing	6	1(16.7)	59	16(27.1)	-
Colon polyps present					
Yes	13	2(15.4)	66	12(18.2)	1.71(0.49-6.04)
No	23	21(91.3)	113	5(4.42)	3.21(0.31-33.50)
Missing	27	1(3.70)	185	31(16.8)	-
Loss of mismatch repair protein expression by IHC					
Yes	10	0(0)	45	0(0)	-
No	37	1(2.70)	229	19(8.30)	1.53(0.28-8.30)
Missing	16	4(25.0)	90	29(32.2)	-
Number of first-degree relatives with cancer diagnosis					
≤ 1	52	4(7.69)	267	32(12.0)	1.37(0.54-3.47)
> 1	2	0(0)	47	3(6.38)	-
Missing	9	1(11.1)	50	13(26.0)	-

P-value: Statistically significant results (in bold).

Cox regression model analysis of parameters associated with OS

We have carefully collated relevant clinical data from TCGA-COAD to investigate the potential risk factors affecting survival in colon cancer patients. Univariate Cox regression analysis revealed statistically significant correlations between clinical stage ($HR = 2.294$, $P = 0.004$), M stage ($HR = 3.554$, $P < 0.001$), residual tumor ($HR = 9.644$, $P < 0.001$), number of examined lymph nodes ($HR = 0.468$, $P = 0.049$), venous invasion ($HR = 2.528$, $P = 0.003$), loss of mismatch repair protein expression by IHC ($HR = 2.9 \times 10^{-9}$, $P = 0.003$), *MK5-AS1* ($HR = 1.106$, $P = 0.043$), and *MK5* expression ($HR = 1.091$, $P = 0.038$) with the prognosis of colon cancer patients. After including these factors in the multivariate Cox regression model, it was found that clinical stage ($HR = 7.052$, $P = 0.037$), residual tumor ($HR = 7.667$, $P = 0.011$), *MK5-AS1* ($HR = 1.064$, $P = 0.046$), and *MK5* ($HR = 1.904$, $P = 0.029$) could be independent risk factors for colon cancer patients [Table 5].

GO and KEGG analysis

We enriched the co-expressed genes of *MK5-AS1* to explore its biological function. For GO “Biological Processes”, the above genes were mainly involved in cytoplasmic translation, mitochondrial respiratory chain complex III assembly, negative regulation of release of cytochrome from mitochondria, and negative regulation of mTOR signaling. In “Cellular Component”, the cytosolic large ribosomal subunit, cytosolic ribosome, polysomal ribosome, mitochondrion, U12-type spliceosomal complex, and endoplasmic

Table 4. Stratified analysis of the relationship between MAPKAPK5 expression and prognosis of clinical colon cancer patients

Characteristics	MAPKAPK5 low		MAPKAPK5 high		Adjusted HR (high vs. low)
	Cases (N)	Deaths (%)	Cases (N)	Deaths (%)	
Sex					
Male	34	15(44.1)	12	5(41.7)	0.86(0.32-2.29)
Female	32	11(34.3)	10	4(40.0)	1.24(0.37-4.17)
Age					
≤ 44	0	0(0)	1	0(0)	-
45-59	17	6(35.3)	9	4(44.4)	1.97(0.59-6.51)
≥ 60	49	20(40.8)	12	5(41.7)	1.15(0.44-2.96)
Clinical stage					
I, II	45	11(24.4)	10	3(33.3)	1.29(0.32-5.12)
III, IV	21	15(71.4)	12	6(50.0)	
Pathology grade					
I, II	62	22(35.5)	17	7(41.2)	1.19(0.49-2.91)
III, IV	4	4(100.0)	5	2(40.0)	0.21(0.04-1.22)
T					
T1, T2	9	2(22.2)	2	0(0)	0.29(0.01-9.89)
T3, T4	57	24(42.1)	20	9(45.0)	1.05(0.48-2.29)
N					
N0	62	24(38.7)	13	5(38.5)	1.04(0.40-2.70)
N1, N2	4	2(50.0)	9	4(44.4)	0.90(0.16-5.06)
M					
M0	63	23(36.5)	22	9(40.9)	1.12(0.51-2.48)
M1	3	3(100.0)	0	0(0)	-
Total lymph nodes					
≤ 10	52	22(42.3)	15	6(40.0)	0.87(0.36-2.08)
> 10	14	4(28.6)	7	3(42.9)	1.68(0.34-8.27)
Tumor location					
Left hemicolon	31	14(45.2)	8	5(62.5)	2.43(0.75-7.81)
Transverse colon	10	5(50.0)	6	1(16.7)	0.31(0.06-1.57)
Right hemicolon	25	7(28.0)	8	3(37.5)	1.18(0.28-4.93)
Classification					
Invasive lesions	17	5(29.4)	5	2(70.6)	1.33(0.22-8.10)
Protuberant lesions	18	5(27.8)	2	0(0)	0.31(0.02-5.36)
Missing	31	16(51.6)	15	7(48.4)	-

reticulum were significantly co-regulated. The signal pathways enriched in “Molecular Functions” included structural constituents of the ribosome, RNA binding, and cadherin binding [Figure 2E]. The lollipop and path diagrams detailed in Figure 2F and G displayed the pathways enriched by KEGG analysis, including alcoholism, neutrophil extracellular trap formation, systemic lupus erythematosus, GABAergic synapse, viral carcinogenesis, morphine addiction, and tyrosine metabolism, which were associated with metabolic and immune-related diseases.

Validation of the expression pattern of MAPKAPK5 protein by IHC and clinical correlation analysis

Our study included 90 matched pairs of colon adenocarcinoma tissues and adjacent non-tumor tissues. As demonstrated in Figure 3A, MK5 protein expression was analyzed in three representative tissue pairs. Positive immunostaining was mainly located in the nucleus. After the evaluation of the IHC score, high expression of MK5 protein was detected in 60.0% (54/90) of most colon cancer sections and 10% (9/90) of

Table 5. Cox regression analyses of clinicopathologic characteristics associated with OS in TCGA samples

Variable		Univariable		
		HR	95%CI	P
A.				
Gender		0.713	0.407-1.247	0.236
Age		1.892	0.947-3.782	0.061
BMI		1.019	0.369-2.393	0.896
Race	1	Reference		
	2	1.196	0.431-1.726	0.675
	3	8.361	0.647-13.361	0.996
Clinical stage		2.294	1.294-4.067	0.004
T		1.133	0.479-2.684	0.800
N		1.835	0.969-3.473	0.062
M		3.554	1.904-6.635	< 0.001
Residual tumor		9.644	3.368-27.61	< 0.001
Histological type		1.796	0.840-3.842	0.131
Lymph node examined count		0.468	0.219-0.999	0.049
Venous invasion		2.528	1.382-4.624	0.003
Lymphatic invasion		2.460	1.349-4.485	0.003
Synchronous colon cancer present		2.086	0.066-3.502	0.469
History of colon polyps		0.863	0.372-2.001	0.731
Colon polyps present		1.519	0.577-4.546	0.397
Loss of mismatch repair protein expression by IHC		2.9×10^{-9}	0-Inf	0.003
Number of first-degree relatives with cancer diagnosis		1.120	0.152-8.228	0.912
MAPKAPK5-AS1		1.106	1.024-1.064	0.043
MAPKAPK5		1.091	1.076-1.257	0.038
B.				
Clinical stage		7.052	1.117-14.249	0.037
M		1.349	0.301-6.033	0.696
Residual tumor		7.667	1.591-36.945	0.011
Lymph node examined count		4.228	0.035-5.126	0.499
Venous invasion		1.286	0.210-7.875	0.785
Lymphatic invasion		1.649	0.259-10.515	0.597
Loss expression of mismatch repair proteins by IHC		4.700	0-Inf	0.998
<i>Lnc-MAPKAPK5-AS1</i>		1.064	1.029-1.144	0.046
<i>MAPKAPK5</i>		1.904	1.771-3.084	0.029

P-value: Statistically significant results (in bold).

tumor-adjacent tissues [Figure 3B]; the significant upregulation trend was consistent with the results of our previous bioinformatic analysis. Simultaneously, for clinical correlation analysis, *MK5* expression was significantly positively correlated with the pathological grade ($\chi^2 = 44.992$, $P = 0.025$) and N stage ($\chi^2 = 15.915$, $P < 0.001$) [Table 6], indicating that *MK5* may play a role in tumor promotion. Additionally, based on prognostic follow-up information of about 6 years, we found that patients with low expression of *MK5* had a longer survival time than those with high expression of *MK5* [Figure 3C], which further confirmed the cancer-promoting effect of *MK5*.

Table 6. Relationship between clinicopathological characteristics and expression of MAPKAPK5 protein in HCC patients

Characteristics	n	MAPKAPK5 expression		χ^2	P-value
		High (n = 22)	Low (n = 66)		
Sex				0.061	0.805
Male	46	12(26.1)	34(73.9)		
Female	42	10(23.8)	32(76.2)		
Age				5.206	0.074
≤ 44	1	1(100)	0(0)		
45-59	26	9(34.6)	17(65.4)		
≥ 60	61	12(19.7)	49(80.3)		
Clinical stage				3.636	0.057
I, II	55	10(18.2)	45(81.8)		
III, IV	33	12(36.4)	21(63.6)		
Pathology grade				4.992	0.025
I, II	79	17(21.5)	62(78.5)		
III, IV	9	5(55.6)	4(44.4)		
T				0.312	0.577
T1, T2	11	2(18.2)	9(72.3)		
T3, T4	77	20(26.0)	57(74.0)		
N				15.915	< 0.001
N0	75	13(17.3)	62(82.7)		
N1, N2	13	9(69.2)	4(30.8)		
M				1.035	0.309
M0	85	22(25.9)	63(74.1)		
M1	3	0(0)	3(100.0)		
Total lymph nodes				16.475	< 0.001
≤ 10	29	15(51.7)	14(48.3)		
> 10	59	7(11.9)	52(88.1)		
Tumor location				1.762	0.414
Left hemicolon	39	8(20.5)	31(79.5)		
Transverse colon	16	6(37.5)	10(62.5)		
Right hemicolon	33	8(24.2)	25(75.8)		
Classification				3.881	0.144
Invasive lesions	23	5(22.7)	18(77.3)		
Protuberant lesions	19	2(10.5)	17(89.5)		
Missing	46	15(32.6)	31(67.4)		

P-value: Statistically significant results (in bold).

PPI network of MAPKAPK5

As shown in [Figure 3D](#), proteins named *MAPK6*, *PARK7*, *HSPB1*, *MAPK4*, *MAPKAPK2*, *NEDD4*, *BCL7B*, *CDCA8*, and *CSNK1E* remarkably interact with *MK5*. *MAPK6* has been reported as the target gene of miRNAs related to colon adenoma carcinogenesis, not only for the early diagnosis of CRC, but also to provide evidence for its carcinogenic mechanism^[20]. Jin proposed that *PARK* is involved in the activation of various intracellular signaling pathways involved in tumor progression, affecting tumor proliferation, metastasis, recurrence, and drug resistance^[21]. In addition, the translation of *MAPK4* mRNA is inhibited by *IGF2BP1*, which further inhibits its phosphorylation by *MK5*, leading to increased cell adhesion and thus promoting cancer cell migration. The above evidence indirectly suggests that *MK5* may influence cell cycle regulation and biological signaling in specific physiological states through these interactions.

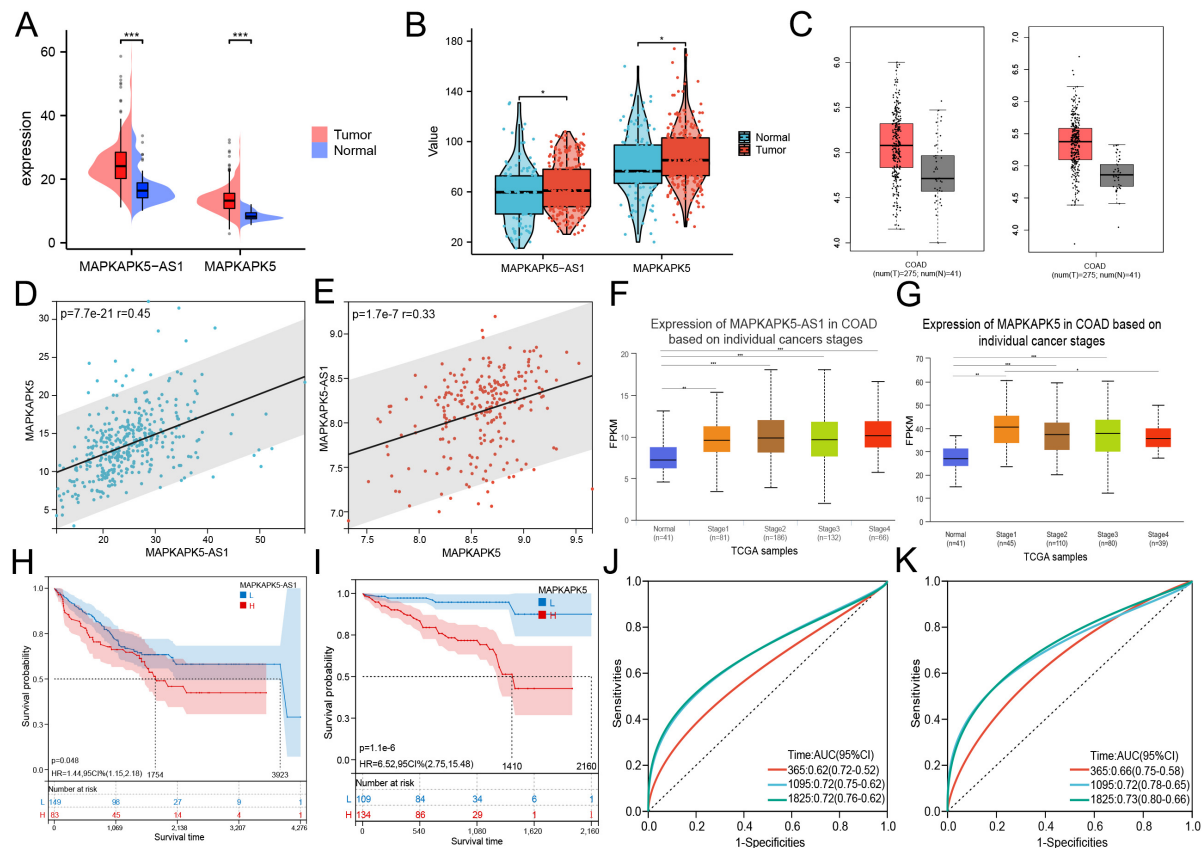


Figure 1. Differential expression of *MK5-AS1* and *MK5* in colon cancer compared to normal tissues and clinical prognosis analysis. (A-C) Comparison of *MK5-AS1* and *MK5* expression in colon cancer tissues and normal tissues based on TCGA-COAD, GSE41258, and GEPIA 2.0. (D and E) Significant positive correlations were observed between *MK5-AS1* and *MK5* expression in TCGA-COAD patients and GSE41258. (F and G) Expression status of *MK5-AS1* and *MK5* in patients with different clinical stages based on the UALCAN database. (H-K) Prognostic analysis of *MK5-AS1*, *MK5* and ROC curves of corresponding 1, 3, and 5-year survival rates. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Gene mutation analysis

As shown in Figure 4A and B, the columns represent samples, while the icons and colors reflect different types of genetic mutations. The mutation frequencies of *MK5-AS1* and *MK5* in the colon cancer dataset named “CPTAC-2 Prospective ($n = 110$)” were 9% and 8%, respectively. Most of the mutations were deep deletions. Furthermore, the Chi-square test was employed to assess the frequency of gene mutation between high and low gene expression groups. The waterfall maps showed 20 genes with significant differences between the two groups, of which 288 samples were tested for mutation, 271 of which were mapped samples (94.1%). The mutation frequency of *APC* and *KRAS* was higher in the *MK5-AS1* high expression group than in the low expression group, while *BRAF*, *RYR3*, *VWF*, and *ZNF469* accounted for a greater proportion of mutations in the *MK5-AS1* low expression group [Figure 4C]. For *MK5*, *APC* also showed a higher mutation frequency in the high expression group, whereas *ZFHX4*, *MUC5B*, *BRAF*, *NEB*, and *KMT2B* displayed the opposite mutation trends [Figure 4D]. To comprehensively characterize the genomic landscape associated with *MK5-AS1* expression, we analyzed TP53 mutation patterns across *MK5-AS1* expression subgroups. Despite observable trends, no statistically significant difference was observed between the *MK5-AS1* high expression group and low expression group ($P > 0.05$). This suggests that TP53 mutational status alone may not directly explain the divergent clinical or molecular phenotypes observed between *MK5-AS1* subgroups. Nevertheless, potential regulatory interactions between TP53 and *MK5-AS1* at transcriptional or post-transcriptional levels warrant further investigation, given the well-documented context-dependent oncogenic effects of TP53 mutations in colorectal carcinogenesis.

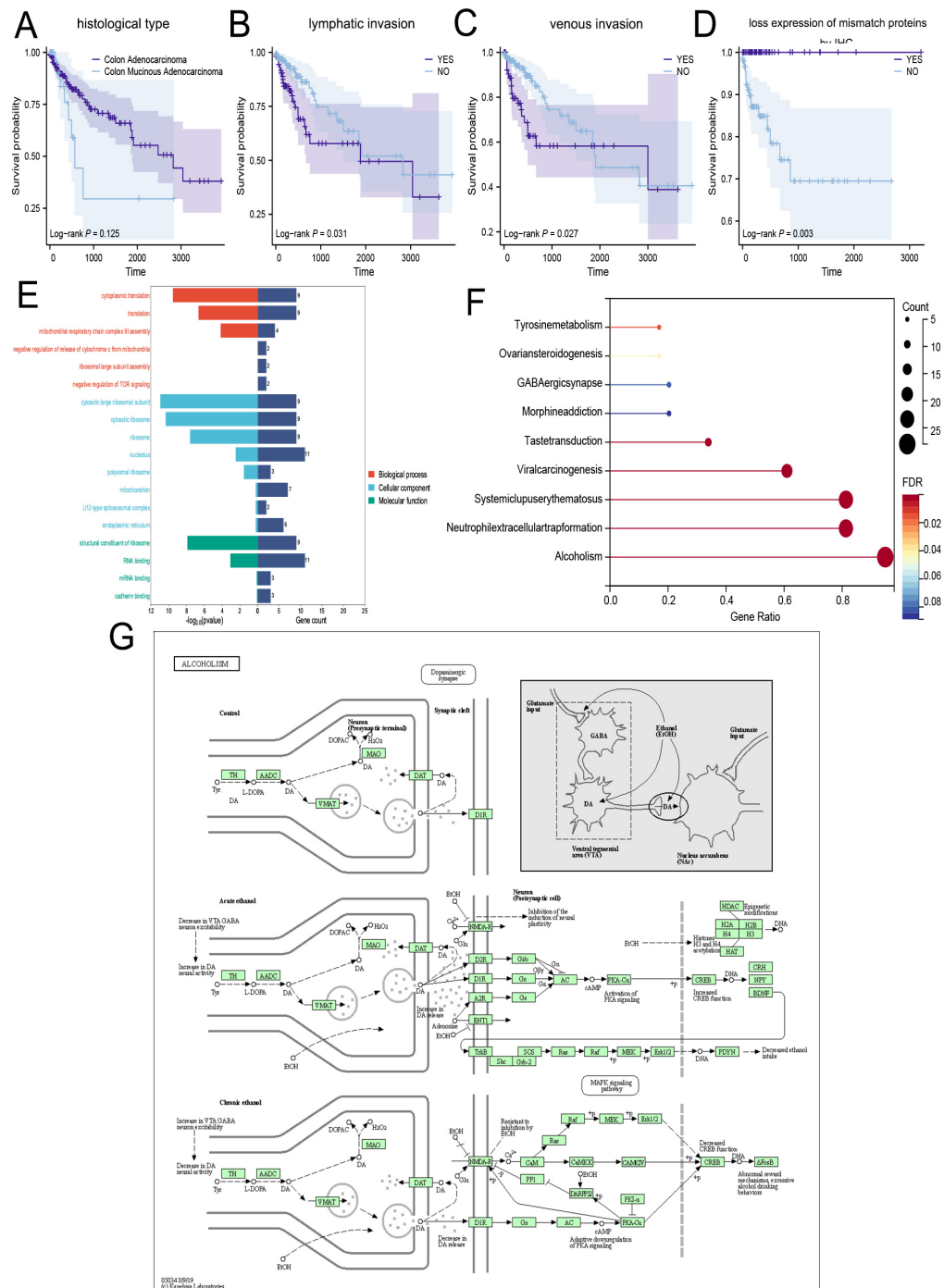


Figure 2. Potential biological processes involved in *MK5-AS1* co-expression genes. (A-D) Survival analysis based on main clinical features. (E and F) GO and KEGG analysis. (G) KEGG pathway: Alcoholism.

Lnc-MAPKAPK5-AS1 is overexpressed in colorectal liver metastases and co-regulated with immune and metabolic pathways

In addition to discussing the role of *MK5-AS1* in colon cancer development and clinical prognosis, we also intended to investigate its potential effect on colon cancer liver metastasis. After normalization with the expression profile of the GEO datasets, we found that both *MK5-AS1* and *MK5* were significantly

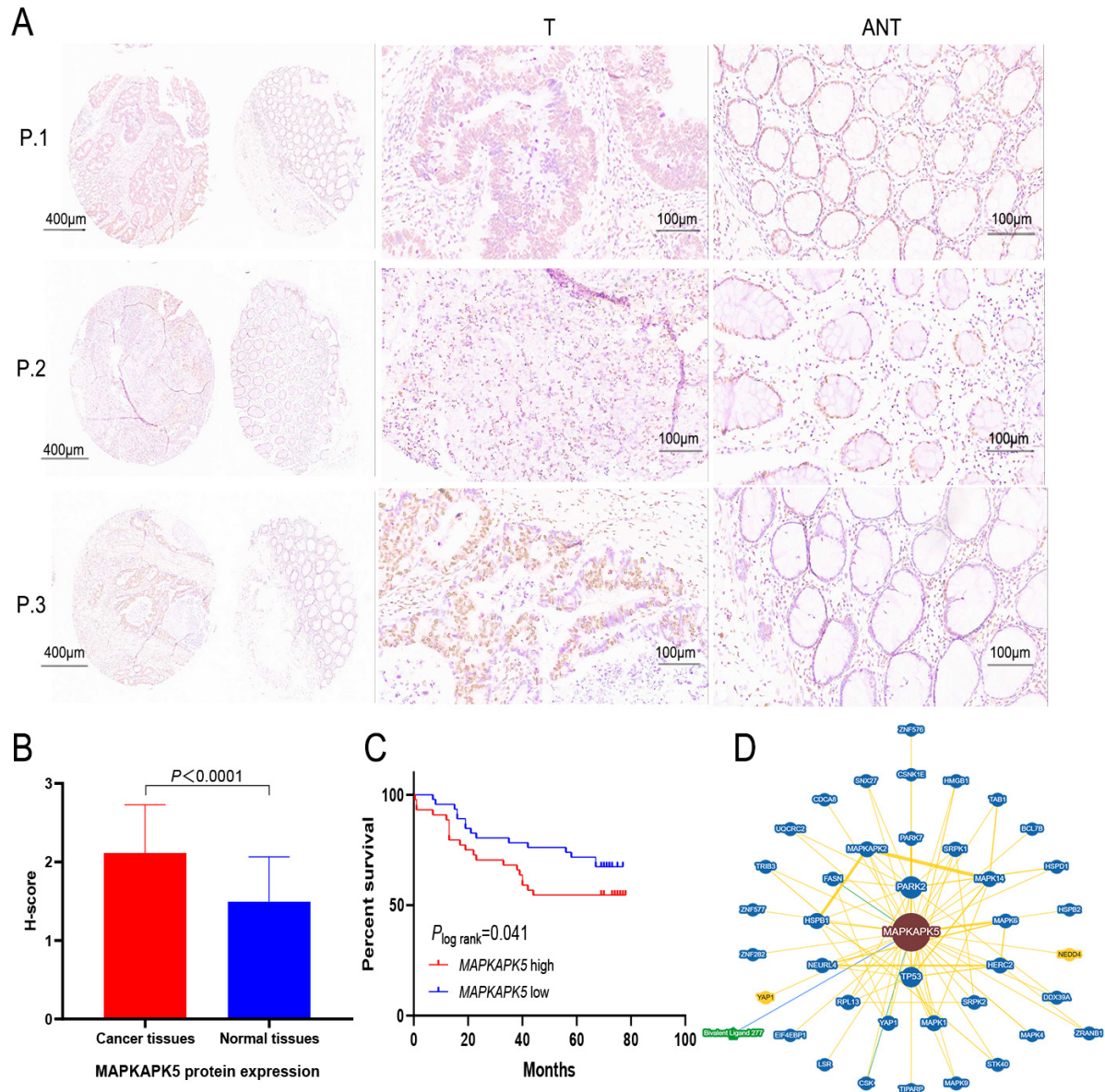


Figure 3. Validation of MAPKAPK5 protein expression by IHC and survival analysis. (A) Expression of MK5 protein in three representative pairs of colon cancer tissues (T) and adjacent non-cancerous tissues (ANT). (B) The MK5 protein expression score is higher in colon cancer tissues compared to para-carcinoma tissues. (C) The relationship between MK5 expression and OS in colon cancer patients. (D) PPI network of MK5 protein based on the BioGRID database.

upregulated in liver metastatic tissues compared to normal colon cancer tissues ($P < 0.001$, $P = 0.035$, respectively; Figure 5A and B). As shown in Figure 5C, the intersection of DEGs between the above two types of tissues in the three GEO datasets was obtained by the Venn diagram, and the “heatmap.2” function was applied to plot cluster heat maps containing upregulated and downregulated DEGs in each dataset (GSE49355: upregulated: 164 genes, downregulated: 107 genes; GSE41258: upregulated: 83 genes, downregulated: 299 genes; GSE18549: upregulated: 88 genes, downregulated: 99 genes), with adjusted P value < 0.05 and $|\text{Log2-FC}| > 1$ [Figure 5D-F].

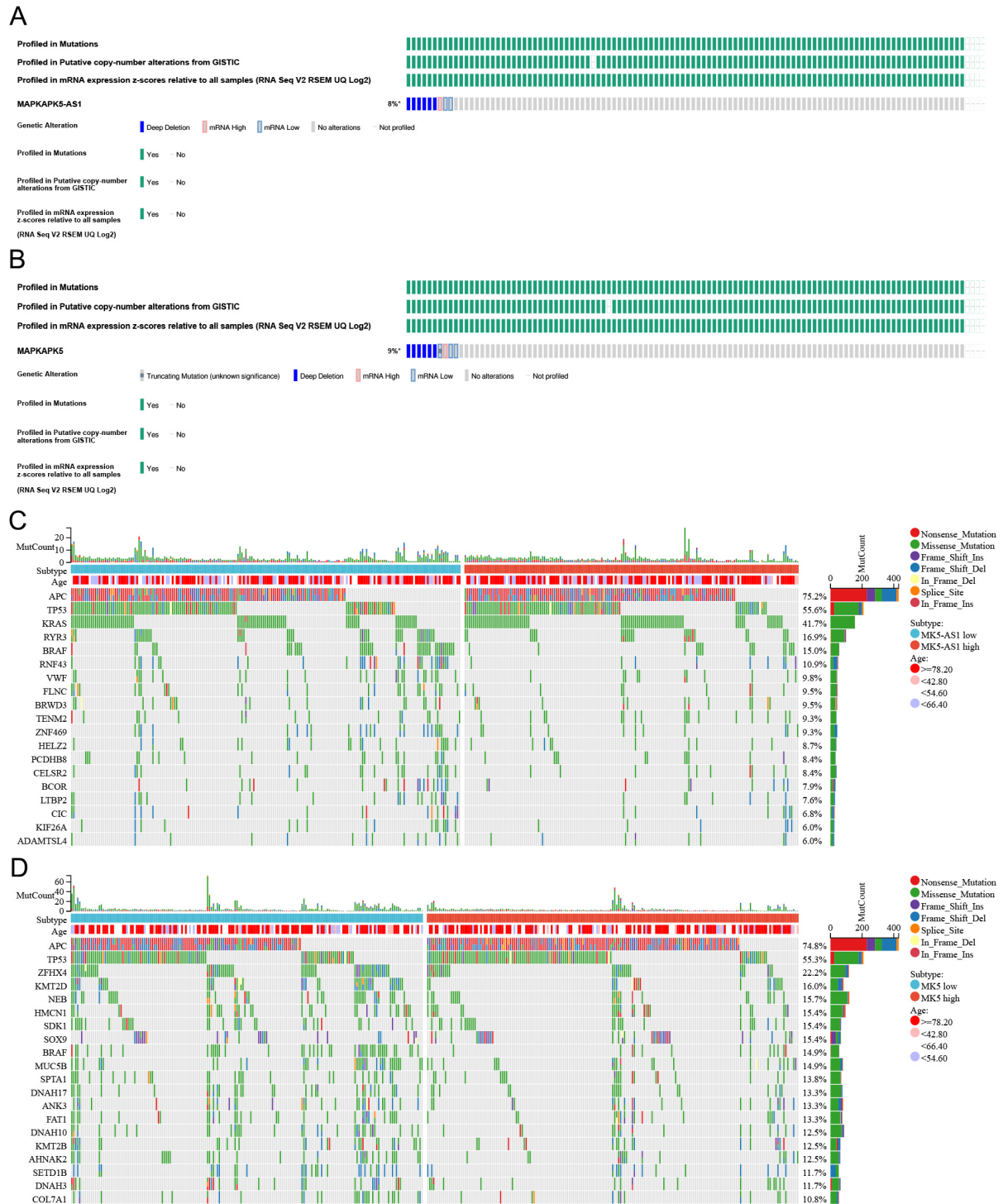


Figure 4. Gene mutation analysis. (A and B) OncoPrint plots of *MK5-AS1* and *MK5* in the cBioPortal database. (C and D) The top 20 genes with the highest mutation frequency in the high and low expression groups of *MK5-AS1* and *MK5*.

We discovered that the genes co-expressed with *MK5-AS1* are involved in immune, inflammatory, and metabolic pathways, suggesting that it may have some effect on colon cancer invasion and metastasis to a certain extent. In GO analysis, upregulated DEGs were mainly involved in blood coagulation, complement

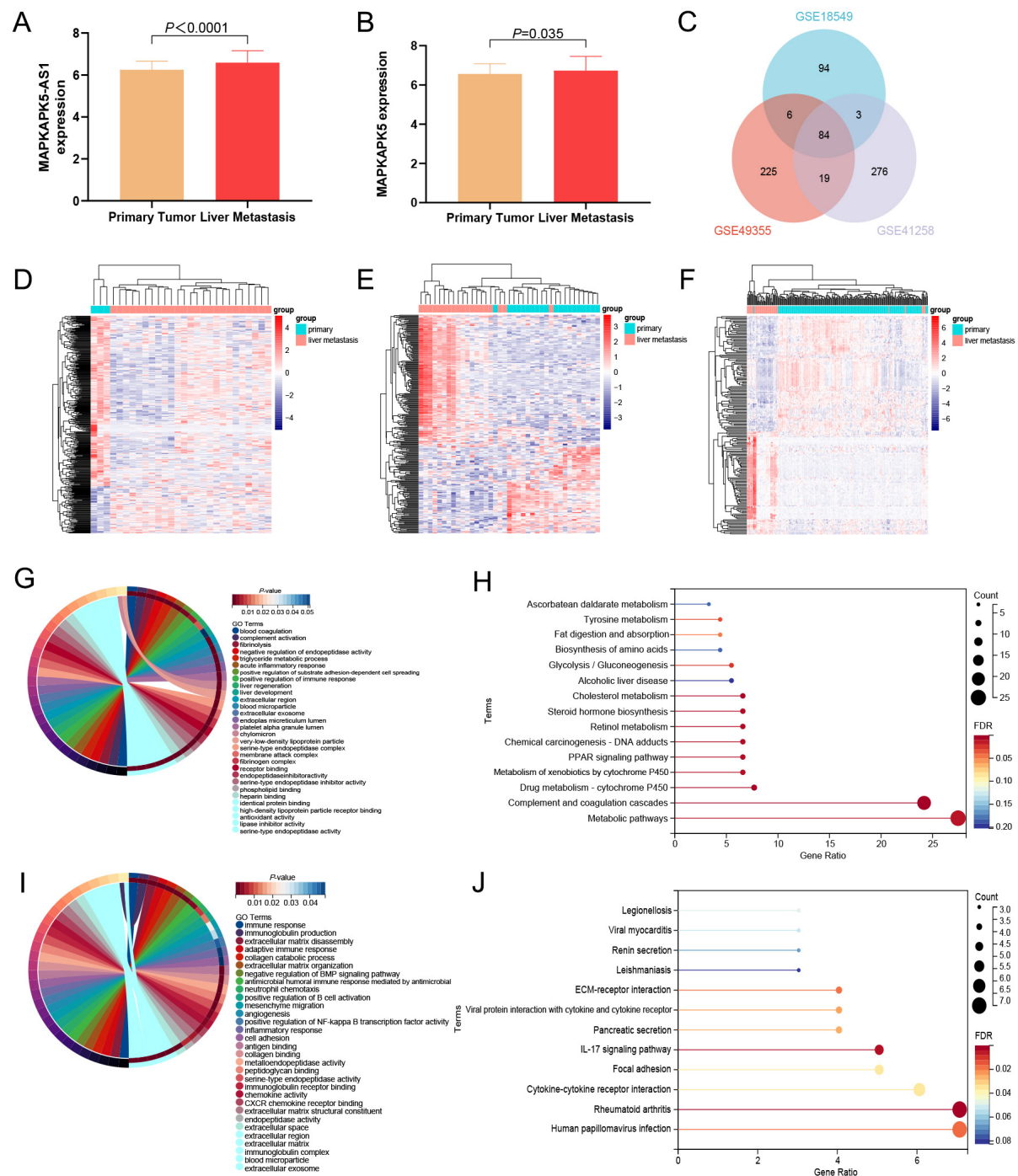


Figure 5. Expression status and functional enrichment analysis of *MK5-AS1* and *MK5* in liver metastasis and colon cancer tissues. (A and B) *MK5-AS1* and *MK5* were significantly upregulated in liver metastasis. (C) Venn diagram showing the intersection of DEGs in three GEO datasets. (D-F) Heat maps of DEGs expression (GSE49355, GSE41258, and GSE18549) in colon cancer and liver metastases. (G and H) GO and KEGG analysis of upregulated genes. (I and J) GO and KEGG analysis of downregulated genes.

activation, fibrinolysis, liver development, acute inflammatory response, positive regulation of immune response, very-low-density lipoprotein particle assembly, negative regulation of very-low-density lipoprotein particle clearance, cholesterol metabolic process, and hemoglobin metabolic process

[Figure 5G]. In KEGG analysis, upregulated DEGs were mainly involved in metabolic pathways, complement and coagulation cascades, drug metabolism-cytochrome P450, metabolism of xenobiotics by cytochrome P450, PPAR signaling pathway, chemical carcinogenesis-DNA adducts, cholesterol metabolism, and alcoholic liver disease [Figure 5H]. As for downregulated DEGs, significant roles were also identified in GO analysis, which were related to immune response, positive regulation of NF-kappa B transcription factor activity, inflammatory response, cell adhesion, neutrophil chemotaxis, angiogenesis, positive regulation of B cell activation, collagen catabolic process, negative regulation of BMP signaling pathway, extracellular exosome, and blood microparticle [Figure 5I]. In KEGG analysis, downregulated DEGs were associated with human papillomavirus infection, rheumatoid arthritis, cytokine-cytokine receptor interaction, focal adhesion, IL-17 signaling pathway, and viral protein interaction with cytokine and cytokine receptors [Figure 5J].

Correlation of gene expression with the proportion of tumor-infiltrating cells

Given our above findings that colon cancer tissues with high *MK5-AS1* expression are enriched in inflammatory immune-related activities, we hypothesized that this might be related to the tumor immune microenvironment. As shown in Figure 6A-C, significant negative correlations were observed between *MK5-AS1* expression and three immune-related scores. Figure 6D and E shows the proportions of 22 various immune cell types in primary colon cancer tissues and liver metastases, with each column representing a different patient. Furthermore, we found that the expression patterns of eight common immune checkpoint genes (*CD274*, *CTLA4*, *PDCD1*, *HAVCR2*, *LAG3*, *PDCD1LG2*, *SIGLEC15*, and *TIGIT*) differed statistically between the *MK5-AS1* high and low expression groups [Figure 6F]. In addition, using two algorithms, “CIBERSORT” and “xCell”, we examined the differences in the abundance of 22 and 64 immune cells between *MK5-AS1* high and low expression groups, respectively [Figure 6G and H]. The results indicated that the proportions of CD4 naive T cells, macrophages M2, dendritic cells, neutrophils, aDC, basophils, cDC, HSC, hepatocytes, MSC, fibroblasts, preadipocytes, and microenvironment score were higher in the low *MK5-AS1* expression group than in the high expression group. On the contrary, regulatory T cells (Tregs), gamma delta T cells, macrophages M0, activated eosinophils, CD8 naive T cells, epithelial cells, osteoblasts, plasma cells, and platelets were higher in the *MK5-AS1* high expression group.

DISCUSSION

The incidence of colon cancer is increasing year by year and tends to be younger, which is closely linked to factors such as genetics, diet, underlying intestinal diseases, and living habits^[22-24]. The prognosis of colon cancer mainly depends on tumor stage and early intervention, while colonoscopy is still difficult to promote in the population. Currently, molecular markers for the diagnosis of colon cancer include carbohydrate antigen (CA), carcinoembryonic antigen (CEA), carbohydrate antigen (CA19-9), etc.^[25,26]. Although these markers can help detect colon cancer, their sensitivity and specificity are low. With the rapid development of molecular genomics, the identification of new regulatory factors from tumors as biomarkers for disease diagnosis and prognosis may provide a solid basis for improving traditional diagnosis and treatment. lncRNAs exert a regulatory role in various biological processes such as embryonic development and gene expression^[27,28]. In recent years, the mechanism of lncRNAs in colon cancer has been widely explored^[29]. For example, compelling studies have constructed promising ferroptosis^[30], cuproptosis^[31], or immune-related^[32] lncRNA signatures to predict the prognosis and response to treatment of colon cancer. While a limited number of lncRNAs have been confirmed to be associated with colorectal cancer metastasis, further exploration is necessary to determine their clinical applications, such as in tumor diagnosis and treatment. Colorectal cancer-associated transcripts (CCATs) have been identified as factors associated with colorectal cancer metastasis and are categorized into two distinct types: CCAT1 and CCAT2. Aberrant expression of CCAT1 has been detected during the progression of colorectal cancer, including the development of lymph node and liver metastases. Increased expression of CCAT2 has been observed in microsatellite-stabilized

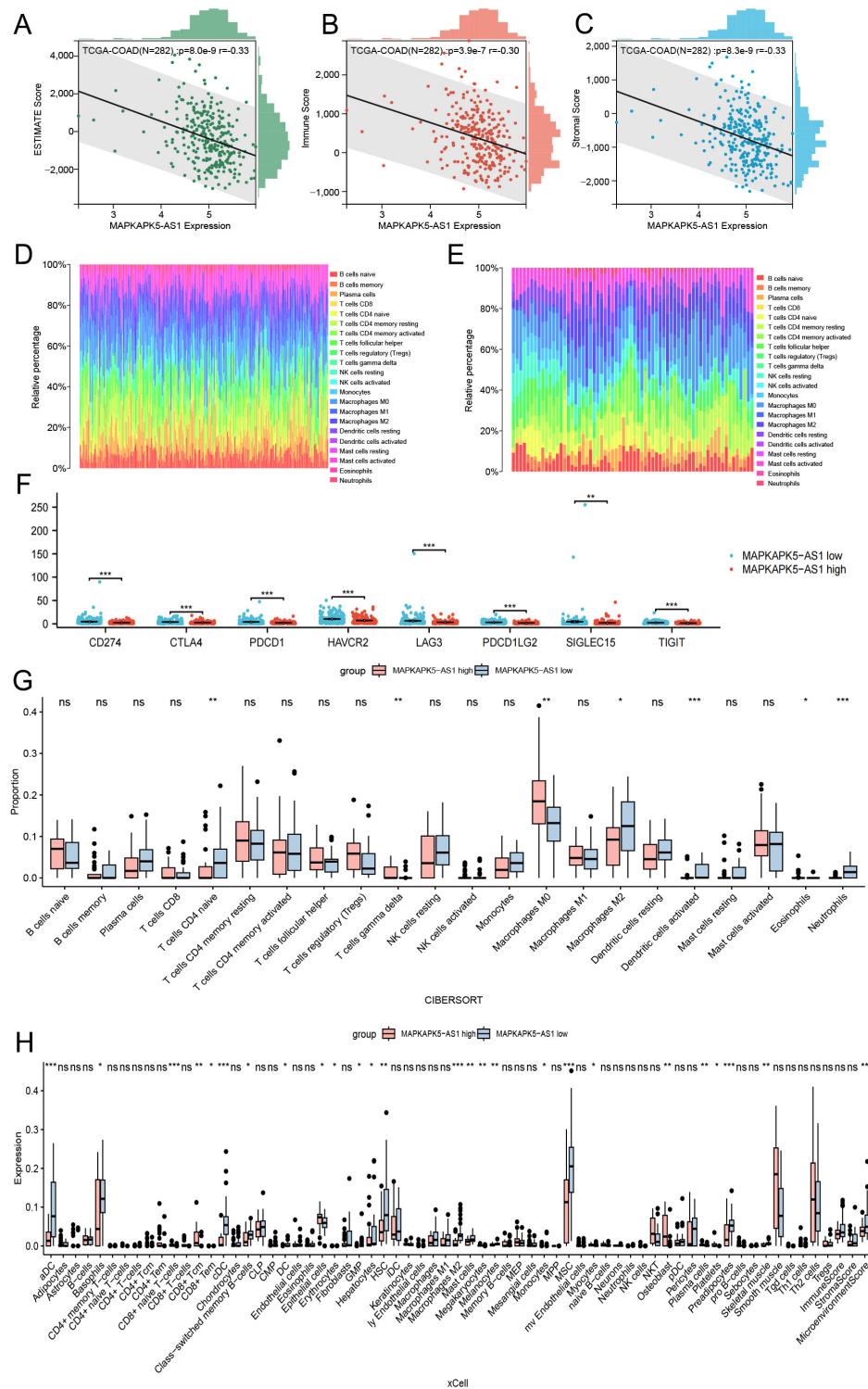


Figure 6. Correlation analysis between *MK5-AS1* and immune microenvironment. (A-C) The relationships between *MK5-AS1* and ESTIMATE score, Immune score, and Stromal score. (D and E) The proportions of 22 types of immune cells in colon cancer tissues and liver metastasis. (F) Expression patterns of eight immune checkpoint genes between the *MK5-AS1* high expression group and the low expression group. (G) The expression characteristics of 22 immune cell phenotypes were calculated based on the "CIBERSORT" algorithm. (H) The expression of 64 types of immune cells extracted based on the "xCell" algorithm was shown in different groups. Statistical significance is indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns: not significant ($P \geq 0.05$).

colorectal cancers, where it activates the Wnt signalling pathway through its interaction with transcription factors. Consequently, this has led to an elevated risk of hepatic metastasis and an increased number of metastatic nodules.

Although significant progress has been achieved in lncRNA research related to colorectal cancer hepatic metastasis, the functional characterization and mechanistic underpinnings of the majority of lncRNAs in this pathological process remain poorly understood. There is limited evidence that the positive feedback loop between *lnc-CYTOR* and the Wnt/ β -catenin signaling pathway promotes colon cancer metastasis^[33], and *lnc-AFAP1-AS1* promotes the invasion and metastasis of colon cancer cells by participating in epithelial-mesenchymal processes that affect cell adhesion^[34]. In addition, carcinoma-associated fibroblasts (CAFs) promoted the transfer of the common oncogene *H19* in exosomes, leading to the activation of the β -catenin pathway to promote colorectal cancer development, metastasis and drug resistance^[35]. Cheng *et al.* demonstrated that *lnc-C00662* facilitates tumor growth and metastatic spread through a competitive endogenous RNA (ceRNA) mechanism, wherein it sequesters *miR-340-5p* to modulate *CLDN8/IL22* co-expression and subsequently activates the ERK signaling pathway^[36]. Furthermore, overexpression of *SNHG7* significantly enhanced the tumorigenesis and liver metastasis of SW480 cells *in vivo*^[37]. As an innovative lncRNA, *MK5-AS1* has been reported to be associated with a variety of tumor-related characteristics such as hypoxia, immune infiltration, ceRNA axes, and the regulation of expression of other proteins, especially in liver cancer^[38-40].

Through integrative analysis of TCGA and GEO datasets, we identified significantly elevated expression levels of both *MK5-AS1* and *MK5* in colon cancer tissues compared to normal controls. Interestingly, these two molecules demonstrated strong positive co-expression. In addition, clinical correlation analysis showed that *MK5-AS1* expression affected the histological type, clinical stage, N stage, lymphatic invasion, and BMI of colon cancer patients. Our own clinical samples also demonstrated that the staining score of *MK5* was significantly higher in cancer tissues than in adjacent normal tissues, and that gene expression was negatively regulated with clinical stage and N stage. Furthermore, multivariate Cox regression analysis showed that M stage, residual tumor, *MK5-AS1*, and *MK5* were independent risk factors for colon cancer. The discovery of prognostic factors is helpful for clinical decision making: our results found that high expression of *MK5-AS1* is associated with shorter OS and may be a predictor of poor prognosis of colon cancer. To control for confounding factors, we performed a stratified prognostic analysis based on clinical features and found that in the N0 and M0 subgroups, *MK5-AS1* high vs. low expression significantly affected the survival outcome of patients.

Furthermore, to elucidate the potential biological functions of *MK5-AS1*, we conducted comprehensive functional annotation and pathway enrichment analyses of its co-expressed genes. The results suggested that the molecular functions of DEGs were mainly enriched in mitochondria-associated metabolic pathways, negative regulation of mTOR signaling, GABAergic synapse, viral carcinogenesis, and tyrosine metabolism. The mTOR protein signaling pathway is an important therapeutic target for various tumors. As a key regulator of the Akt signaling pathway, mTOR regulates translation and transcription by phosphorylating a variety of downstream proteins, thereby affecting tumorigenesis and metastasis of tumors^[41-43]. While our enrichment analysis suggests a potential role of *MK5-AS1* in lipid/amino acid metabolism, these findings require further experimental validation. Future studies should employ metabolomics and functional assays to directly assess *MK5-AS1*'s metabolic impact and identify underlying mechanisms.

Based on 110 colon cancer samples collected online from the cBioPortal database, we found that 9 (8%) of all samples had a deep deletion of *MK5-AS1*, and 9% of the mutation frequency was present in *MK5*. We also found that there were some differences in mutations between the *MK5-AS1* and *MK5* expression groups. In the high expression group, there was a higher frequency of mutations in genes such as *APC*, *KRAS*, *etc.*, which was consistent with the results of genome-wide analysis by Nakayama^[44]. In primary colon cancer, mutations in the proto-oncogene *KRAS* are most commonly identified, and a significant increase in neutrophils in the blood of patients with *KRAS*-mutated tumors has been found, leading to the recruitment of CLM sites^[45]. Meanwhile, the frequency of the *BRAF* mutation was higher in the low expression group. Kakar *et al.* found that a mutation in this gene was closely associated with high chromosomal instability and resistance to cetuximab and panitumumab in colon cancer^[46]. The lack of a statistically significant difference in TP53 mutation frequency between *MK5-AS1* subgroups suggests that it may influence TP53-independent pathways or cooperate with other high-mutation genes (e.g., *APC*, *KRAS*) to drive tumor progression. Future studies could investigate whether *MK5-AS1* modulates TP53 downstream targets or interacts with mutant TP53 to alter its oncogenic gain-of-function effects.

From an anatomical point of view, the blood from the colon flows directly into the liver, mainly through the portal vein, which means that the liver is the first stop for colon cancer cells. Therefore, the liver is the most common organ for blood metastasis of colon cancer^[47], which is also the reason why we are paying close attention to whether *MK5-AS1* is associated with liver metastasis of colon cancer. As expected, *MK5-AS1* was also shown to be upregulated in liver metastases of colon cancer in three GEO datasets containing liver metastases and tumor tissue *in situ* in our analysis. In addition, the intersection of upregulated and downregulated differentially expressed genes in the three datasets was screened for further enrichment analysis. GO analysis revealed that the upregulated differentially expressed genes were significantly involved in inflammatory pathways, such as acute inflammatory response, complement activation, and fibrinolysis. Downregulated DEGs were mainly involved in immune response pathways and are closely related to cell adhesion, B cell activation, and neutrophil chemotaxis. In the KEGG analysis, DEGs were mainly involved in metabolic pathways, including drug metabolism, cholesterol metabolism, and the PPAR signaling pathway. Studies have shown that cancer cells that penetrate deeply into the liver alter the normal liver microenvironment and are affected by abnormal liver regeneration, inflammation, fibrosis, and other pathophysiological factors, resulting in tumor susceptibility. Changes in the substructure of normal tissue cells provide preconditions such as genetic material, energy, and proteins for the micrometastasis of the original tumor within the organ^[48].

Given our identification of several immunological pathways associated with *MK5-AS1* in liver metastases and primary cancer tissues, we then investigated its association with the immune microenvironment of colon cancer. Using the ESTIMATE algorithm, we computed immune scores for colon cancer patients, revealing an inverse correlation between *MK5-AS1* expression levels and immune scores. Baldin *et al.* evaluated the clinicopathological features and immune scores of 221 patients with different preoperative treatments for CRLM and found that patients with high immune scores had the lowest risk of recurrence^[49]. In the study by Wang *et al.*, 249 patients with CRLM were included and the prognosis was stratified by combining the pathology score and the immune score, and it was also found that high immune score was associated with a better prognosis^[50]. In the general trend, the degree of immune infiltration represents an important prognostic factor, and a high abundance of tumor-infiltrating lymphocytes has been shown to be associated with improved survival in patients with primary colon cancer. However, the application of immunotherapy in colon cancer is still in the preliminary stage of exploration, and the identification of tumor microenvironment characteristics of colon cancer may provide more reliable predictive markers for the precise implementation of clinical immunotherapy. Comparative analysis revealed significant

differences in immune cell infiltration patterns between primary colon tumors and their matched liver metastases. The presence of more immunosuppressive cells in the liver may partly explain why immunotherapy is less effective in patients with liver metastases^[51], providing new insights into the potential role of *MK5-AS1* in tumor immunology to some extent.

To further investigate the association between *MK5-AS1* and TME immune cell subtypes, “ssGSEA” and “xCell” algorithms were applied to determine the composition of immune cells in each sample of the *MK5-AS1* high and low expression groups. The infiltration of naive B cells, NK cells, and CD8⁺ T cells was associated with patient prognosis^[52]. It was found that the infiltration abundance of regulatory T cells, macrophages Mo, gamma delta T cells, and CD8⁺ naive T cells was higher in the high expression group. These findings suggest *MK5-AS1* might play a potential role in the recruitment, infiltration, and functional regulation of inflammatory and immune cells in the TME, further confirming the importance of exploring the interaction between tumor and immune cells. *MK5-AS1* has also been demonstrated to exert an influence on the balance of the tumor immune microenvironment by modulating the proliferation, differentiation, and function of immune cells^[53]. For example, it promotes the proliferation and activity of certain immunosuppressive cells, thereby suppressing anti-tumor immune responses. On the other hand, it may reduce immune surveillance and clearance in the tumor immune microenvironment by inhibiting the function of immune-activating cells such as dendritic cells. Furthermore, *MK5-AS1* may also affect tumor cell growth and proliferation by regulating signaling pathways related to amino acid metabolism and lipid metabolism^[54]. There is evidence that highly expressed *MK5-AS1* can be transferred to HBV⁺HCC cells via M2 macrophage-derived exosomes, thereby promoting the proliferation of HBV⁺HCC cells by targeting c-Myc^[39].

While our current immune infiltration analyses provide valuable preliminary insights into the tumor immune microenvironment associated with *MK5-AS1* expression, we acknowledge that the mechanistic relationship between *MK5-AS1* and immune cell recruitment requires more extensive investigation. Moving forward, we aim to explore potential correlations between *MK5-AS1* expression patterns and key immunomodulatory molecules, including chemokines and cytokines. Subsequent validation through in vitro co-culture systems would help establish causal relationships, while spatial analyses using multiplex immunohistochemistry could reveal the dynamic interplay between *MK5-AS1*-expressing tumor cells and immune infiltrates within the tumor microenvironment. Such comprehensive approaches will be crucial for determining whether *MK5-AS1* modulates immune cell recruitment primarily through chemokine regulation or an alternative biological pathway.

Although our study implicates *MK5-AS1* in colon cancer hepatic metastasis, several limitations warrant consideration, particularly the absence of mechanistic validation through functional studies (e.g., knockout/overexpression experiments or migration/invasion assays), which limits mechanistic insight into how *MK5-AS1* exerts its effects in colon cancer. Second, while bioinformatics analysis revealed correlations between *MK5-AS1* and the tumor microenvironment, these results cannot establish causal relationships. Third, the sample size in immunohistochemistry validation may constrain the generalizability of the conclusions. In addition, it should be noted that the TCGA-COAD dataset, despite its breadth across disease stages, exhibits incomplete representation of certain molecular subtypes and less prevalent stages due to sample size constraints. Future studies with larger cohorts and functional experiments are needed to validate and expand upon these observations.

Our findings establish *MK5-AS1* as a promising diagnostic and prognostic biomarker for colon cancer, offering distinct advantages over current methods. In clinical practice, this biomarker could be utilized in

several key applications: first, standardized detection assays could enable non-invasive monitoring through liquid biopsies, facilitating early diagnosis and disease surveillance. Second, when combined with conventional clinicopathological parameters, *MK5-AS1* expression levels may enhance prognostic accuracy by improving risk stratification for metastasis and treatment response. Third, the biomarker's dynamic expression patterns could serve as a valuable tool for tracking disease progression and therapeutic efficacy in real time. These efforts will focus particularly on high-risk outcomes like liver metastasis and cancer-related mortality. By addressing these key challenges through prospective and retrospective cohort studies, we aim to optimize the clinical utility and facilitate the integration of *MK5-AS1* into personalized treatment strategies for colon cancer patients. The observed correlations with liver metastasis and immune dysregulation suggest targetable pathways, but key challenges remain: the precise mechanistic role in tumor microenvironment modulation needs elucidation, RNA-based therapeutics face delivery and specificity hurdles, and current associations require validation through *in vivo* models to establish causality.

Conclusion

Integrative bioinformatics analysis of public colon cancer datasets identified *MK5-AS1* as a putative prognostic biomarker and therapeutic target, with its elevated expression significantly correlated with both tumor microenvironment remodeling and liver metastasis. These findings warrant further experimental validation.

DECLARATIONS

Acknowledgments

The authors thank all participants involved in the IHC study of this research.

Authors' contributions

Conceptualization and investigation: Wu D, Hu X

Methodology: Tang K, Wu D, Wang D, Chen J

Data curation: Hu X, Yan Q

Writing - review & editing: Tang K, Wu D

Availability of data and materials

The data that support the findings of this study are openly available in online databases mentioned in the manuscript, and are also available from the corresponding author upon reasonable request.

Financial support and sponsorship

This work was funded by the National Natural Science Foundation of China (81803325), Natural Science Foundation of Guangdong (2021A1515011175, 2024A1515011646), Guangzhou Science and Technology Project (202102080126), The Key Project of Medicine Discipline of Guangzhou (2025-2027-12), the Medical Science and Technology Foundation of Guangdong (A2024733), and Basic Research Project of Key Laboratory of Guangzhou (202102100001, 202206080008).

Conflicts of interest

Wu D is a Junior Editorial Board member of *Journal of Cancer Metastasis and Treatment*. Wu D was not involved in any steps of editorial processing, notably including reviewer selection, manuscript handling, or decision making, while the other authors have declared that they have no conflicts of interest.

Ethical approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and the use of IHC was approved by the ethics committee of Shanghai Outdo Biotech Co., Ltd (The approval code of the ethics committee is

SHYJS-CP-1901001).

Consent for publication

Not applicable.

Copyright

© The Author(s) 2025.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71:209-49. DOI
2. Devesa SS, Chow W. Variation in colorectal cancer incidence in the united states by subsite of origin. *Cancer.* 1993;71:3819-26. DOI PubMed
3. Engstrand J, Nilsson H, Strömberg C, Jonas E, Freedman J. Colorectal cancer liver metastases - a population-based study on incidence, management and survival. *BMC Cancer.* 2018;18:78. DOI PubMed PMC
4. Xu R, Wang W, Zhu B, et al. Disease characteristics and treatment patterns of Chinese patients with metastatic colorectal cancer: a retrospective study using medical records from China. *BMC Cancer.* 2020;20:131. DOI PubMed PMC
5. Liu SJ, Dang HX, Lim DA, Feng FY, Maher CA. Long noncoding RNAs in cancer metastasis. *Nat Rev Cancer.* 2021;21:446-60. DOI PubMed PMC
6. Chen S, Shen X. Long noncoding RNAs: functions and mechanisms in colon cancer. *Mol Cancer.* 2020;19:167. DOI PubMed PMC
7. Wu K, Xu T, Song X, et al. LncRNA SLCO4A1-AS1 modulates colon cancer stem cell properties by binding to miR-150-3p and positively regulating SLCO4A1. *Lab Invest.* 2021;101:908-20. DOI PubMed PMC
8. Chen Q, Zhou L, Ma D, et al. LncRNA GAS6-AS1 facilitates tumorigenesis and metastasis of colorectal cancer by regulating TRIM14 through miR-370-3p/miR-1296-5p and FUS. *J Transl Med.* 2022;20:356. DOI PubMed PMC
9. Yang T, Chen WC, Shi PC, et al. Long noncoding RNA MAPKAPK5-AS1 promotes colorectal cancer progression by cis-regulating the nearby gene MK5 and acting as a let-7f-1-3p sponge. *J Exp Clin Cancer Res.* 2020;39:139. DOI PubMed PMC
10. Ji H, Hui B, Wang J, et al. Long noncoding RNA MAPKAPK5-AS1 promotes colorectal cancer proliferation by partly silencing p21 expression. *Cancer Sci.* 2019;110:72-85. DOI PubMed PMC
11. Gao GF, Parker JS, Reynolds SM, et al. Before and after: comparison of legacy and harmonized TCGA genomic data commons' data. *Cell Syst.* 2019;9:24-34.e10. DOI PubMed PMC
12. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets-update. *Nucleic Acids Res.* 2013;41:D991-5. DOI PubMed PMC
13. Oughtred R, Stark C, Breitkreutz BJ, et al. The BioGRID interaction database: 2019 update. *Nucleic Acids Res.* 2019;47:D529-41. DOI PubMed PMC
14. Sherman BT, Hao M, Qiu J, et al. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res.* 2022;50:W216-21. DOI PubMed PMC
15. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* 2017;45:W98-102. DOI PubMed PMC
16. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal.* 2013;6:p11. DOI PubMed PMC
17. Shen W, Song Z, Zhong X, et al. Sangerbox: a comprehensive, interaction-friendly clinical bioinformatics analysis platform. *Imeta.* 2022;1:e36. DOI PubMed PMC
18. Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. *Genome Biol.* 2017;18:220. DOI PubMed PMC
19. Chandrashekar DS, Karthikeyan SK, Korla PK, et al. UALCAN: an update to the integrated cancer data analysis platform. *Neoplasia.* 2022;25:18-27. DOI PubMed PMC
20. Shi Y, Zhuang Y, Zhang J, Chen M, Wu S. Identification of tumorigenic and prognostic biomarkers in colorectal cancer based on microRNA expression profiles. *Biomed Res Int.* 2020;2020:7136049. DOI PubMed PMC
21. Jin W. Novel insights into PARK7 (DJ-1), a potential anti-cancer therapeutic target, and implications for cancer progression. *J Clin Med.* 2020;9:1256. DOI PubMed PMC
22. Willett W. The search for the causes of breast and colon cancer. *Nature.* 1989;338:389-94. DOI PubMed
23. Hofseth LJ, Hebert JR, Chanda A, et al. Early-onset colorectal cancer: initial clues and current views. *Nat Rev Gastroenterol Hepatol.* 2020;17:352-64. DOI PubMed PMC
24. Degirolamo C, Modica S, Palasciano G, Moschetta A. Bile acids and colon cancer: solving the puzzle with nuclear receptors. *Trends Mol Med.* 2011;17:564-72. DOI PubMed
25. Konishi T, Shimada Y, Hsu M, et al. Association of preoperative and postoperative serum carcinoembryonic antigen and colon cancer outcome. *JAMA Oncol.* 2018;4:309-15. DOI PubMed PMC

26. Nakamura Y, Shida D, Tanabe T, et al. Prognostic impact of preoperatively elevated and postoperatively normalized carcinoembryonic antigen levels following curative resection of stage I-III rectal cancer. *Cancer Med.* 2020;9:653-62. DOI PubMed PMC
27. Zhao H, Hu S, Qi J, et al. Increased expression of HOXA11-AS attenuates endometrial decidualization in recurrent implantation failure patients. *Mol Ther.* 2022;30:1706-20. DOI PubMed PMC
28. Pan X, Li C, Feng J. The role of lncRNAs in tumor immunotherapy. *Cancer Cell Int.* 2023;23:30. DOI PubMed PMC
29. Garrett WS. The gut microbiota and colon cancer. *Science.* 2019;364:1133-5. DOI PubMed
30. Wu Z, Lu Z, Li L, et al. Identification and validation of ferroptosis-related lncRNA signatures as a novel prognostic model for colon cancer. *Front Immunol.* 2021;12:783362. DOI PubMed PMC
31. Xu M, Mu J, Wang J, Zhou Q, Wang J. Construction and validation of a cuproptosis-related lncRNA signature as a novel and robust prognostic model for colon adenocarcinoma. *Front Oncol.* 2022;12:961213. DOI PubMed PMC
32. Li X, Yang L, Wang W, Rao X, Lai Y. Constructing a prognostic immune-related lncRNA model for colon cancer. *Medicine.* 2022;101:e30447. DOI
33. Yue B, Liu C, Sun H, et al. A positive feed-forward loop between lncRNA-CYTOR and Wnt/ β -catenin signaling promotes metastasis of colon cancer. *Mol Ther.* 2018;26:1287-98. DOI PubMed PMC
34. Bo H, Fan L, Li J, et al. High expression of lncRNA AFAP1-AS1 promotes the progression of colon cancer and predicts poor prognosis. *J Cancer.* 2018;9:4677-83. DOI PubMed PMC
35. Yang Q, Wang X, Tang C, Chen X, He J. [Retracted] H19 promotes the migration and invasion of colon cancer by sponging miR-138 to upregulate the expression of HMGA1. *Int J Oncol.* 2021;58:26. DOI PubMed PMC
36. Cheng B, Rong A, Zhou Q, Li W. lncRNA LINC00662 promotes colon cancer tumor growth and metastasis by competitively binding with miR-340-5p to regulate CLDN8/IL22 co-expression and activating ERK signaling pathway. *J Exp Clin Cancer Res.* 2020;39:5. DOI PubMed PMC
37. Shan Y, Ma J, Pan Y, Hu J, Liu B, Jia L. lncRNA SNHG7 sponges miR-216b to promote proliferation and liver metastasis of colorectal cancer through upregulating GALNT1. *Cell Death Dis.* 2018;9:722. DOI PubMed PMC
38. Wang L, Sun L, Liu R, et al. Long non-coding RNA MAPKAPK5-AS1/PLAGL2/HIF-1 α signaling loop promotes hepatocellular carcinoma progression. *J Exp Clin Cancer Res.* 2021;40:72. DOI PubMed PMC
39. Tao L, Li D, Mu S, Tian G, Yan G. lncRNA MAPKAPK5-AS1 facilitates cell proliferation in hepatitis B virus-related hepatocellular carcinoma. *Lab Invest.* 2022;102:494-504. DOI
40. Lv E, Sheng J, Yu C, Rao D, Huang W. Long noncoding RNA MAPKAPK5-AS1 promotes metastasis through regulation miR-376b-5p/ECT2 axis in hepatocellular carcinoma. *Dig Liver Dis.* 2023;55:945-54. DOI
41. Meng S, Jian Z, Yan X, Li J, Zhang R. lncRNA SNHG6 inhibits cell proliferation and metastasis by targeting ETS1 via the PI3K/AKT/mTOR pathway in colorectal cancer. *Mol Med Rep.* 2019;20:2541-8. DOI PubMed PMC
42. Lin S, Wang H, Yang W, Wang A, Geng C. Silencing of long non-coding RNA colon cancer-associated transcript 2 inhibits the growth and metastasis of gastric cancer through blocking mTOR signaling [Retraction]. *Oncotargets Ther.* 2022;15:1507-8. DOI PubMed PMC
43. Cai Q, Zhou W, Wang W, et al. MAPK6-AKT signaling promotes tumor growth and resistance to mTOR kinase blockade. *Sci Adv.* 2021;7:eabi6439. DOI PubMed PMC
44. Nakayama M, Oshima M. Mutant p53 in colon cancer. *J Mol Cell Biol.* 2019;11:267-76. DOI PubMed PMC
45. Polidoro MA, Milana F, Soldani C, et al. Impact of RAS mutations on the immune infiltrate of colorectal liver metastases: a preliminary study. *J Leukoc Biol.* 2020;108:715-21. DOI
46. Kakar S, Deng G, Sahai V, et al. Clinicopathologic characteristics, CpG island methylator phenotype, and BRAF mutations in microsatellite-stable colorectal cancers without chromosomal instability. *Arch Pathol Lab Med.* 2008;132:958-64. DOI
47. Bertocchi A, Carloni S, Ravenda PS, et al. Gut vascular barrier impairment leads to intestinal bacteria dissemination and colorectal cancer metastasis to liver. *Cancer Cell.* 2021;39:708-24.e11. DOI
48. Wang H, Zhang B, Li R, et al. KIAA1199 drives immune suppression to promote colorectal cancer liver metastasis by modulating neutrophil infiltration. *Hepatology.* 2022;76:967-81. DOI
49. Baldin P, Van den Eynde M, Mlecnik B, et al. Prognostic assessment of resected colorectal liver metastases integrating pathological features, RAS mutation and Immunoscore. *J Pathol Clin Res.* 2021;7:27-41. DOI PubMed PMC
50. Wang Y, Lin HC, Huang MY, et al. The immunoscore system predicts prognosis after liver metastasectomy in colorectal cancer liver metastases. *Cancer Immunol Immunother.* 2018;67:435-44. DOI PubMed PMC
51. Zhou SN, Pan WT, Pan MX, et al. Comparison of immune microenvironment between colon and liver metastatic tissue in colon cancer patients with liver metastasis. *Dig Dis Sci.* 2021;66:474-82. DOI
52. Schaafsma E, Jiang C, Cheng C. B cell infiltration is highly associated with prognosis and an immune-infiltrated tumor microenvironment in neuroblastoma. *J Cancer Metastasis Treat.* 2021;7:34. DOI PubMed PMC
53. Ge J, Tao M, Zhang G, Cai J, Li D, Tao L. New HCC subtypes based on CD8 tex-related lncRNA signature could predict prognosis, immunological and drug sensitivity characteristics of hepatocellular carcinoma. *J Hepatocell Carcinoma.* 2024;11:1331-55. DOI
54. Ni H, Wang XS, Diener K, Yao Z. MAPKAPK5, a novel mitogen-activated protein kinase (MAPK)-activated protein kinase, is a substrate of the extracellular-regulated kinase (ERK) and p38 kinase. *Biochem Biophys Res Commun.* 1998;243:492-6. DOI PubMed