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Mechanistic insights into FEN1-mediated drug sensitivity and risk signature in colon cancer

An integrative bioinformatics study

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Abstract

The overexpression of Flap endonuclease 1 (FEN1) has been implicated in drug resistance and prognosis across various cancer types. However, the precise role of FEN1 in colon cancer remains to be fully elucidated. In this study, we employed comprehensive datasets from The Cancer Genome Atlas, Gene Expression Omnibus, and Human Protein Atlas to examine FEN1 expression and assess its correlation with clinical pathology and prognosis in colon cancer. We utilized the pRRophetic algorithm to evaluate drug sensitivity and performed differential expression analysis to identify genes associated with FEN1-mediated drug sensitivity. Gene set enrichment analysis was conducted to further investigate these genes. Additionally, single-cell sequencing analysis was employed to explore the relationship between FEN1 expression and functional states. Cox regression analysis was implemented to construct a prognostic model, and a nomogram for prognosis was developed. Our analysis of The Cancer Genome Atlas and Gene Expression Omnibus datasets revealed a significant upregulation of FEN1 in colon cancer. However, while FEN1 expression showed no notable correlation with prognosis, it displayed associations with metastasis. Single-cell sequencing analysis further confirmed a positive correlation between FEN1 expression and colon cancer metastasis. Furthermore, we detected marked discrepancies in drug responsiveness between the High_FEN1 and Low_FEN1 groups, identifying 342 differentially expressed genes. Enrichment analysis showed significant suppression in processes related to DNA replication, spliceosome, and cell cycle pathways in the Low_FEN1 group, while the calcium signaling pathway, cAMP signaling pathway, and other pathways were activated. Of the 197 genes differentially expressed and strongly linked to FEN1 expression, 39 were significantly implicated in colon cancer prognosis. Finally, we constructed a risk signature consisting of 5 genes, which, when combined with drug treatment and pathological staging, significantly improved the prediction of colon cancer prognosis. This study offers novel insights into the interplay among FEN1 expression levels, colon cancer metastatic potential, and sensitivity to therapeutic agents. Furthermore, we successfully developed a multi-gene prognostic risk signature derived from FEN1.

Abbreviations: FEN1 = Flap endonuclease 1, TCGA = The Cancer Genome Atlas.

Keywords: colon cancer, drug resistance, FEN1, metastasis, prognosis

1. Introduction

Colon cancer ranks third worldwide in terms of its incidence and second in terms of cancer-related mortality. It is projected that by 2020, there will be more than 1.9 million new cases of colon cancer and 935,000 deaths, representing approximately one-tenth of all cancer cases and deaths.[1] Substantial research endeavors have focused on uncovering the etiology, preventive strategies, and treatment modalities for colon cancer. Age, family history, colorectal polyps, inflammatory bowel disease, dietary habits, and obesity are recognized risk factors.[2,3] Genetic mutations and genomic variations play pivotal roles in the initiation and progression of colon cancer, with mutations in genes such as APC, KRAS, and TP53 commonly observed in colon cancer patients.[4–6] Furthermore, dysbiosis of the intestinal microbiota has been closely associated with the pathogenesis and advancement of colon cancer.[7] A comprehensive understanding of colon cancer, achieved through rigorous research endeavors, is fundamental in enabling early detection, personalized therapeutic interventions, and the formulation of effective preventive measures.

The Flap endonuclease 1 (FEN1) gene encodes a pivotal endonuclease that plays a crucial role in maintaining genome stability and facilitating DNA repair processes.[8] The FEN1 protein holds significant importance in the realm of DNA replication and repair, particularly in its ability to process DNA

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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flap structures found at the termini of DNA fragments.[9–11] Recent investigations have shed light on the substantial involvement of the FEN1 gene in a diverse range of malignancies. Mutations or aberrant expression of the FEN1 gene have been closely associated with vital biological activities within tumor cells, including heightened proliferation, invasive tendencies, and resistance to therapeutic interventions.[12–14] Moreover, the polymorphic variations observed within the FEN1 gene have demonstrated correlations with individual predisposition to cancer and treatment responsiveness.[15] Consequently, a comprehensive exploration of the FEN1 gene holds remarkable significance in advancing our comprehension of tumorigenesis mechanisms, facilitating the development of innovative therapeutic approaches, and realizing the potential of individualized treatment modalities.

Notwithstanding, the precise implications and effects of FEN1 in terms of prognostication and chemotherapy response in the context of colon cancer have yet to be conclusively established. This study endeavors to systematically unravel the intricate expression patterns of FEN1 within colon cancer via comprehensive bioinformatics analyses, thereby shedding light on its prognostic significance. Furthermore, our objective encompasses an in-depth investigation into the FEN1-mediated drug sensitivity in colon cancer, thereby unraveling the intricate molecular mechanisms that underlie this phenomenon. Lastly, we aspire to construct a robust multi-gene prognostic signature that holds predictive value for patients with colon cancer.

2. Materials and methods

2.1. FEN1 expression analysis

The TCGA-COAD cohort comprised 514 colon cancer patients, encompassing 473 tumor samples and 41 adjacent normal tissues was obtained from The Cancer Genome Atlas (TCGA) database. This cohort was accompanied by comprehensive clinical prognostic data. Additionally, the GSE38939 and GSE44861 datasets, retrieved from the gene expression omnibus database, were employed to validate the expression patterns of FEN1 in both normal and colon cancer tissues. To further corroborate the expression profile of FEN1, immunohistochemical staining was performed using the Human Protein Atlas database for assessing FEN1 expression levels in colon cancer and normal colon tissues.

2.2. Drug sensitivity analysis

In the TCGA-COAD cohort, patients were stratified into high-expression and low-expression groups based on the median expression value of FEN1. The drug sensitivity for different group was analyzed using the "pRRophetic" R package.[16]

2.3. Differential expression and gene set enrichment analysis

Differential expression analysis was conducted to identify genes exhibiting significant expression disparities between the high and low FEN1 expression groups. This analysis was executed utilizing the "limma" package, using adjusted P-value < 0.05 and |log(fold change)| > 1.2 as thresholds for selecting differentially expressed genes.[17] Gene Ontology and Kyoto Encyclopedia of Genes and Genomes gene set enrichment analysis was conducted using the "clusterProfiler" R package to unravel the underlying biological functions and signaling pathways associated with FEN1.[18]

2.4. Single-cell transcriptomic analysis

We employed the CancerSEA website (http://biocc.hrbmu.edu.cn/CancerSEA)[19] to investigate the correlation between FEN1 expression and cancer-related functional states, encompassing processes such as angiogenesis, apoptosis, cell cycle progression, et al.

2.5. Construction and assessment of prognostic models

Univariate Cox regression analysis was conducted to identify differentially expressed genes associated with the prognosis of colon cancer. Genes with a P-value < 0.05 were selected for subsequent Lasso and multivariate Cox regression analyses to construct prognostic features using the "glmnet" package.[20] The risk score was calculated using the formula: riskscore = Σ(coef i × expi), where coefi represents the corresponding coefficient and expi represents the gene expression level. The TCGA-COAD cohort was stratified into high-risk and low-risk groups based on the median riskscore. Survival disparities between the groups were evaluated using Kaplan–Meier survival curves and log-rank tests. Receiver operating characteristic curves were used to assess the predictive performance of the riskscore.

2.6. Construction and evaluation of nomograms

Both univariate and multivariate Cox regression models were applied to quantify the prognostic association between the riskscore and clinical-pathological features in colon cancer. The "rms"[21] package was employed to construct a nomogram and generate calibration curves, decision curves were generated using "rmda".[22]

2.7. Statistical analysis

Statistical analysis and visualization were conducted using SPSS software (version 20.0, SPSS Inc., Chicago, IL) and R software (version 4.2.2).[23] Chi-square tests were used to assess the differences in clinical variables between High_FEN1 and Low_FEN1 groups. Statistical significance was defined as a two-tailed P-value < 0.05.

3. Results

3.1. Expression of FEN1 and its prognostic significance

Through an analysis of Pan-Cancer data obtained from TCGA, we identified a notable upregulation of FEN1 expression across several crucial cancer types (Fig. 1A). Consequently, our research endeavors were directed towards elucidating the specific role of FEN1 in colon cancer. More precisely, our investigation revealed a substantial increase in FEN1 expression within COAD tissues (P < .001; Fig. 1B). To validate these findings, we further examined the expression patterns of FEN1 using the gene expression omnibus datasets GSE38939 (n = 19 per group) and GSE44861 (n = 55 per group). The results indicated a comparatively lower expression of FEN1 in tumor tissues when compared to their corresponding normal controls (Fig. 1C and D). Immunohistochemical analysis using data from the human protein atlas database revealed a pronounced expression of the MPP7 protein in tumor tissues, while negligible expression was observed in normal tissues like endothelial cells and peripheral nerve/ganglion. Notably, heightened expression of FEN1 was specifically detected in glandular cells (Fig. 1E). Nonetheless, survival analysis did not reveal a statistically significant difference in

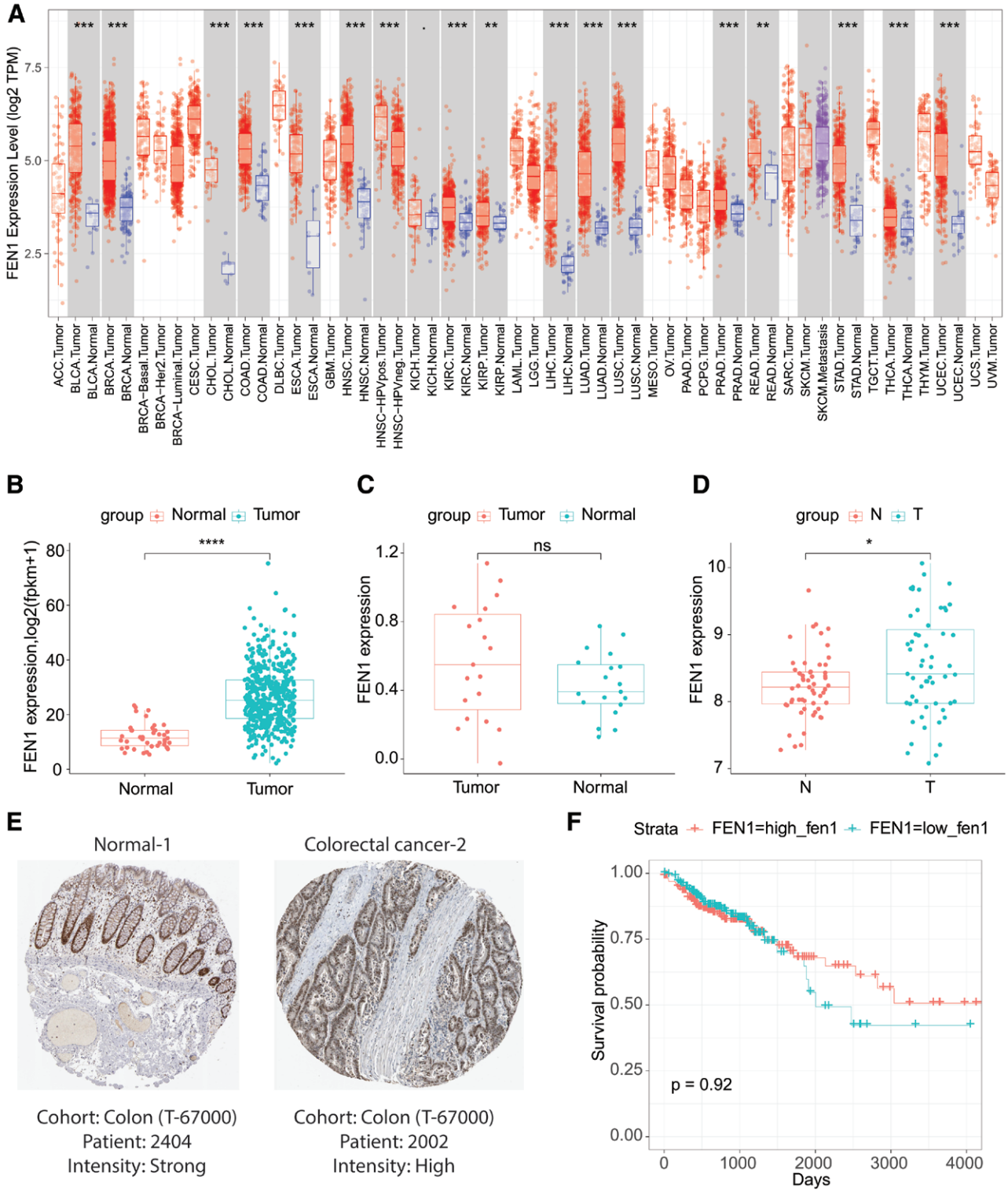


Figure 1. Aberrant expression of FEN1 in colon cancer tumors and adjacent tissues. (A) Expression levels of FEN1 in 26 types of cancers and normal tissues (TCGA cancer data vs TCGA normal data). (B) Expression levels of FEN1 in TCGA-COAD cohort for cancer versus adjacent tissues. (C and D) Expression patterns of FEN1 in samples from GSE38939 (n = 19 per group) and GSE44861 (n = 55 per group) datasets. (E) Validation of FEN1 expression levels in colon cancer and normal tissues using IHC staining from the HPA database. (F) Survival analysis plots for the High_FEN1 and Low_FEN1 subsets. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$; ns, $P > .05$. FEN1 = flap endonuclease 1, HPA = human protein atlas, TCGA = The Cancer Genome Atlas.

overall survival between patients with high versus low FEN1 expression (Fig. 1F). The included colon cancer patients from the TCGA-COAD cohort were subsequently categorized into high (n = 198) and low (n = 198) FEN1 expression groups. A comprehensive assessment of baseline characteristics, as presented in Table 1, revealed a noteworthy association between the expression levels of FEN1 and the M stage of colon cancer ($P < .026$). Specifically, elevated FEN1 expression correlated with an increased frequency of patients categorized as stage M1.

Table 1

The baseline characteristics of patients with CRC according to the expression levels of FEN1 in TCGA-COAD cohort.

Characteristics	Low FEN1	High FEN1	*P*-value
Sample size	198	198	
T stage, n (%)			.445
T1	6	3	
T2	26	33	
T3	146	137	
T4	20	25	
N stage, n (%)			.695
N0	107	114	
N1	52	45	
N2	39	39	
M stage, n (%)			.026
M0	153	114	
M1	22	36	
MX	23	18	
Pathologic stage, n (%)			.279
I	33	38	
II	74	85	
III	64	54	
IV	31	21	
Gender, n (%)			.131
Female	114	99	
Male	84	99	
Age (years), n (%)			.328
>60	132	141	
≤60	66	57	
Outcomes, n (%)			.613
Dead	37	41	
Alive	161	157	

FEN1 = flap endonuclease 1, TCGA = The Cancer Genome Atlas.

3.2. FEN1 correlated with metastasis in colon cancer

Based on the comprehensive dataset obtained from the CancerSEA repository, our investigation revealed a lack of significant correlation between the expression levels of FEN1 and established biomarkers of colon cancer (Fig. 2A). Utilizing cutting-edge single-cell sequencing techniques,[24] we meticulously profiled 290 individual cells and employed t-distributed stochastic neighbor embedding visualization to discern their distinct gene expression patterns (Fig. 2B and C). Furthermore, our study demonstrated a noteworthy positive association between FEN1 expression and cellular metastasis in colon cancer, as evidenced by a correlation coefficient of 0.47 ($P < .01$) (Fig. 2D).

3.3. FEN1 involved in drug sensitivity of colon cancer

Considering the crucial role of FEN1 in determining cancer drug sensitivity, we conducted an assessment of drug sensitivity within the TCGA-COAD cohort and compared the disparities between the High_FEN1 and Low_FEN1 groups. Our findings demonstrate substantial variances in drug sensitivity between the 2 groups, with the High_FEN1 cohort displaying significantly diminished responsiveness towards various pharmaceutical agents (Fig. 3A). Differential expression analysis unveiled 99 significantly upregulated genes and 243 significantly downregulated genes in the High_FEN1 group, as compared to the Low_FEN1 group (Fig. 3B). Gene set enrichment analysis further delineated the functional implications of these differentially expressed genes, revealing a notable suppression of biological processes associated with DNA replication while concomitantly activating processes such as complement activation and response to BMP (Fig. 3C). Moreover, crucial pathways including splicesome, DNA replication, and cell cycle were significantly restrained, whereas signaling pathways such as ECM-receptor, phospholipase D, Calcium, and cAMP exhibited pronounced activation (Fig. 3D). Correlation analysis elucidated that among these differentially expressed genes, 197 genes displayed significant co-expression with FEN1, of which 110 genes demonstrated a positive correlation while 87 genes exhibited a negative correlation (Fig. 3E, Table S1, Supplementary Digital Content, http://links.lww.com/MD/L971).

3.4. Risk features derived from differentially expressed genes associated with FEN1

Univariate Cox regression analysis was performed to explore the prognostic significance of the 342 differentially expressed genes, revealing that 39 genes exhibited significant associations with colon cancer prognosis (Table S2, Supplementary Digital Content, http://links.lww.com/MD/L972). Subsequently, using Lasso Cox shrinkage, a subset of 12 genes was identified as the most relevant (Fig. 4A and B). Multivariate Cox regression analysis further confirmed that CALB1, MND1, NRXN1, TCAM1, and TCL6 were independent prognostic genes in colon cancer (Fig. 4C). Based on these findings, a risk score was established utilizing the formula: riskscore = 0.225767 × CALB1 − 5.6917 × MND1 + 3.127128 × NRXN1 + 0.70729 × TCAM1 + 0.856831 × TCL6. By stratifying the TCGA-COAD cohort into high-risk (n = 198) and low-risk (n = 198) groups according to the median riskscore, survival analysis demonstrated a significantly favorable prognosis in the low-risk group compared to the high-risk group ($P = .0074$, Fig. 4D). Assessment of the receiver operating characteristic curves revealed that the riskscore exhibited promising predictive value for 1-year, 3-year, and 5-year overall survival with respective area under the curve values of 0.714, 0.627, and 0.617 among TCGA-COAD patients (Fig. 4E).

3.5. Clinicopathological correlation of risk features derived from FEN1

In order to investigate the association between risk features derived from FEN1 and clinicopathological characteristics, a comprehensive analysis was conducted. The findings depicted in Figure 5 indicated a significant disparity in riskscores between female and male patients, with female patients exhibiting higher riskscores. Furthermore, a notable elevation in riskscores was observed in deceased patients compared to the surviving cohort. However, no significant differences in riskscores were detected among subgroups stratified by factors such as radiation therapy, drug treatment, age, pathological stage, and TNM stage.

3.6. Construction and evaluation of a prognostic nomogram for colon cancer

Univariate Cox analysis demonstrated significant associations between riskscore and pathological stage in relation to clinical prognosis in colon cancer. Through the multivariate analysis, the final model was established, comprising drug treatment, pathological stage, and riskscore as key variables (Fig. 6A and B). By integrating these factors, a nomogram model was developed to effectively predict the 1-year, 3-year, and 5-year survival rates in clinical patients (Fig. 6C). These findings emphasized the pivotal role of riskscore derived from FEN1, in conjunction with other relevant clinical factors, as a leading prognostic indicator for survival in colon cancer patients. Moreover, the prognostic calibration analysis, which incorporated drug treatment, pathological stage, and riskscore, demonstrated remarkable accuracy in predicting the 1-year, 3-year, and 5-year survival rates (Fig. 6D). In addition, decision curve analysis further highlighted the superior net benefit of the nomogram, encompassing drug treatment,

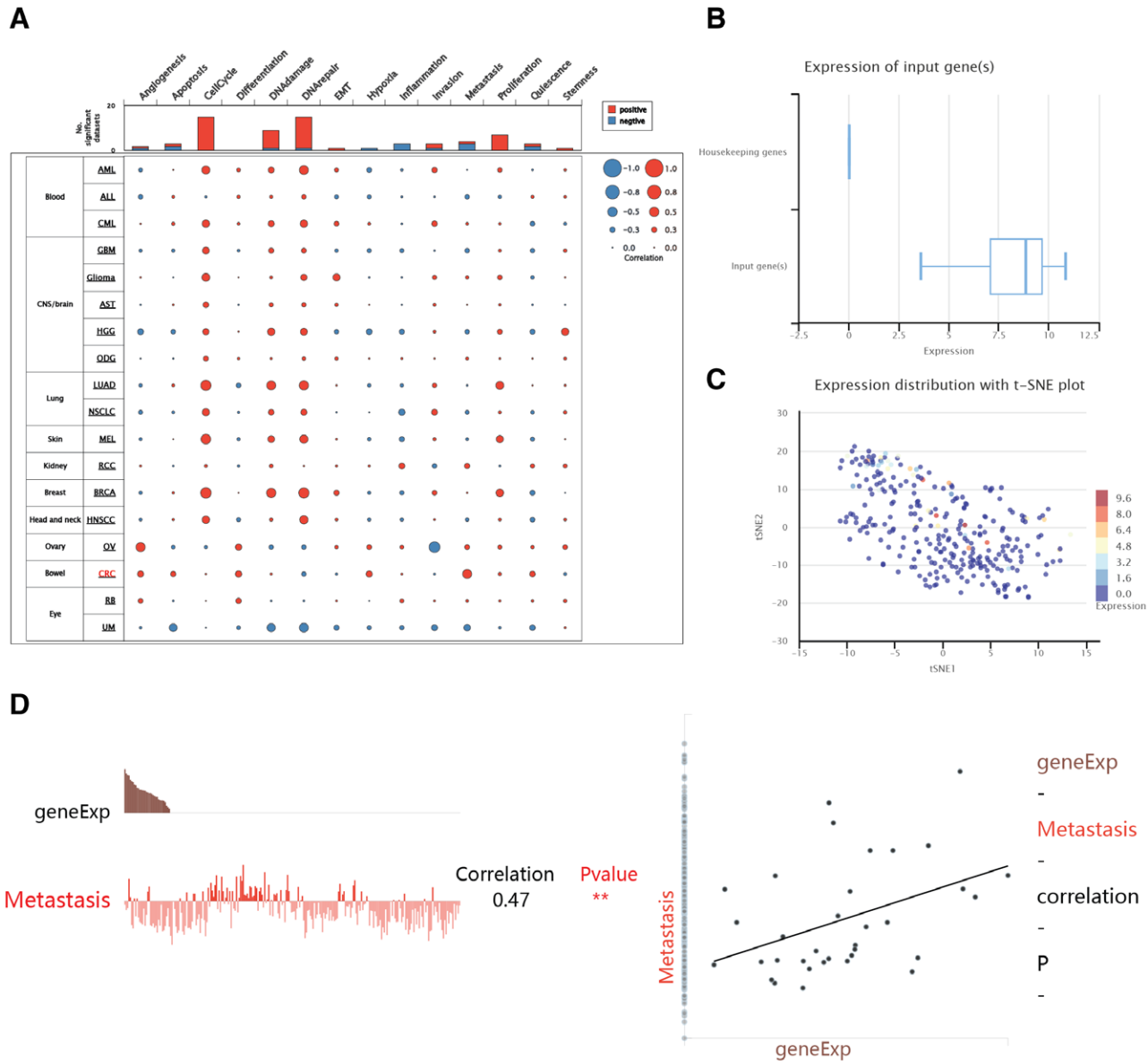


Figure 2. Single-cell sequencing analysis of the functional state of FEN1 in colon cancer. (A) CancerSEA database reveals the relationship between cancer hallmarks and FEN1 expression. (B–D) A specific single-cell sequencing study focused on the role of FEN1 in colon cancer. $^{**}P < .01$. FEN1 = flap endonuclease 1.

pathological stage, and riskscore, in predicting the 1-year overall survival when compared to alternative factors (Fig. 6E).

4. Discussion

Acknowledging the pivotal contribution of FEN1 to tumor progression and chemoresponsiveness, our study employs comprehensive bioinformatics methodologies to address the current knowledge deficit concerning its role in colorectal cancer progression and chemotherapy sensitivity. Although the correlation between FEN1 and colon cancer prognosis did not yield statistical significance, the integration of data from TCGA and single-cell sequencing consistently revealed the involvement of FEN1 in colon cancer metastasis. Prior studies have also implicated a relationship between FEN1 and metastasis in breast cancer.[25] Mechanistically, FEN1 may promote tumor progression through the Wnt/β-catenin signaling pathway[26] and could potentially establish an immunosuppressive microenvironment via the IFN-γ/JAK/STAT-1 axis.[27] Collectively, these observations reinforce the notion that FEN1 constitutes a compelling therapeutic target for cancer intervention, warranting further experimental validation.

FEN1 has been implicated in drug sensitivity, and targeted inhibition of the ERK/ELK1 signaling pathway can suppress FEN1 expression,[28] thereby promoting DNA damage and enhancing chemotherapeutic responsiveness in prostate cancer cells.[29] Moreover, FEN1 is regulated by IGF-1R/miR-610 and mediates chemotherapy sensitivity in osteosarcoma.[30] However, the mechanisms underlying FEN1-mediated drug sensitivity remain poorly understood. Our results confirm differential drug sensitivities between high and low FEN1 expression groups and identify 342 differentially expressed genes associated with FEN1-mediated chemotherapy response, among which 197 genes co-express with FEN1. These findings provide new insights into the investigation of FEN1-mediated mechanisms in colorectal cancer metastasis and chemotherapy response. Enrichment analysis revealed the significant suppression of genes related to DNA replication and the cell cycle, while the ECM-receptor, phospholipase D, Calcium, and cAMP signaling pathways were notably activated. These signaling

pathways have been widely implicated in the biological behavior of various cancer cells. For instance, the Calcium signaling pathway plays a crucial role in regulating intracellular calcium (2+) homeostasis, thereby influencing cellular processes such as proliferation, migration, and invasion.[31] The cAMP signaling pathway modulates the biological functions of malignant cells through the activation of protein kinase A and downstream targets, including cAMP response element-binding protein.[32] In addition, phospholipase D serves as a regulator of intercellular signaling and metabolic pathways involved in vesicular transport, exocytosis, autophagy, cellular metabolic regulation, and tumorigenesis, thus offering potential therapeutic targets for cancer treatment.[33] Through further exploration, the intricate interplay between the aberrant expression of FEN1 and these signaling pathways can be elucidated, shedding light on their impact on cancer cells.

Moreover, a multi-gene prognostic signature for colon cancer was constructed based on the aforementioned differentially expressed genes associated with prognosis. The signature comprises 5 genes, namely CALB1, MND1, NRXN1, TCAM1, and TCL6. Previous investigations have demonstrated the tumor suppressor role of CALB1, as it facilitates cancer cell proliferation and colony formation by enhancing the interaction between p53 and MDM2 while impeding senescence.[34] The prognostic utility of this five-gene signature, when integrated with patients' comprehensive clinical profiles including their drug treatment

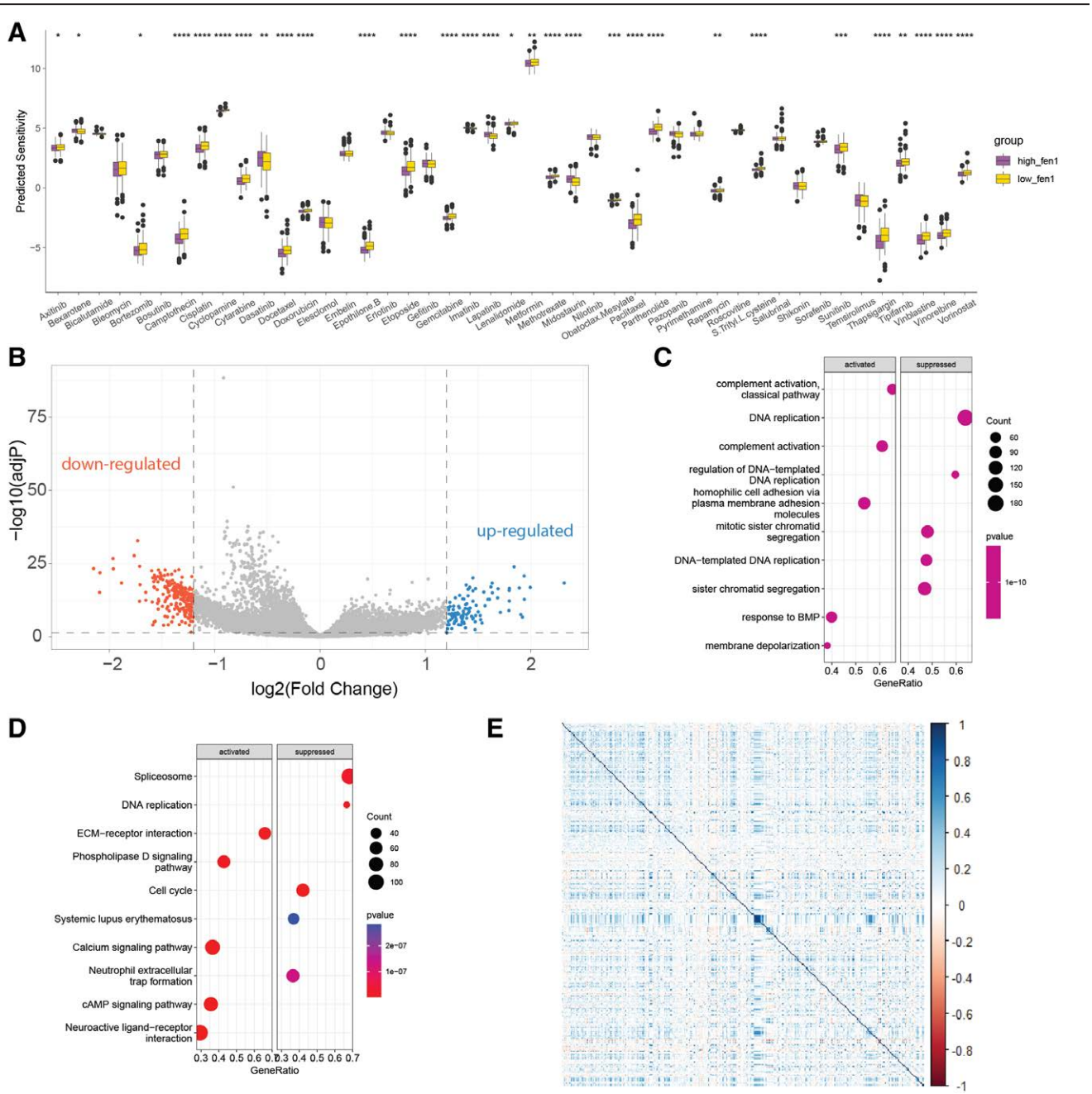
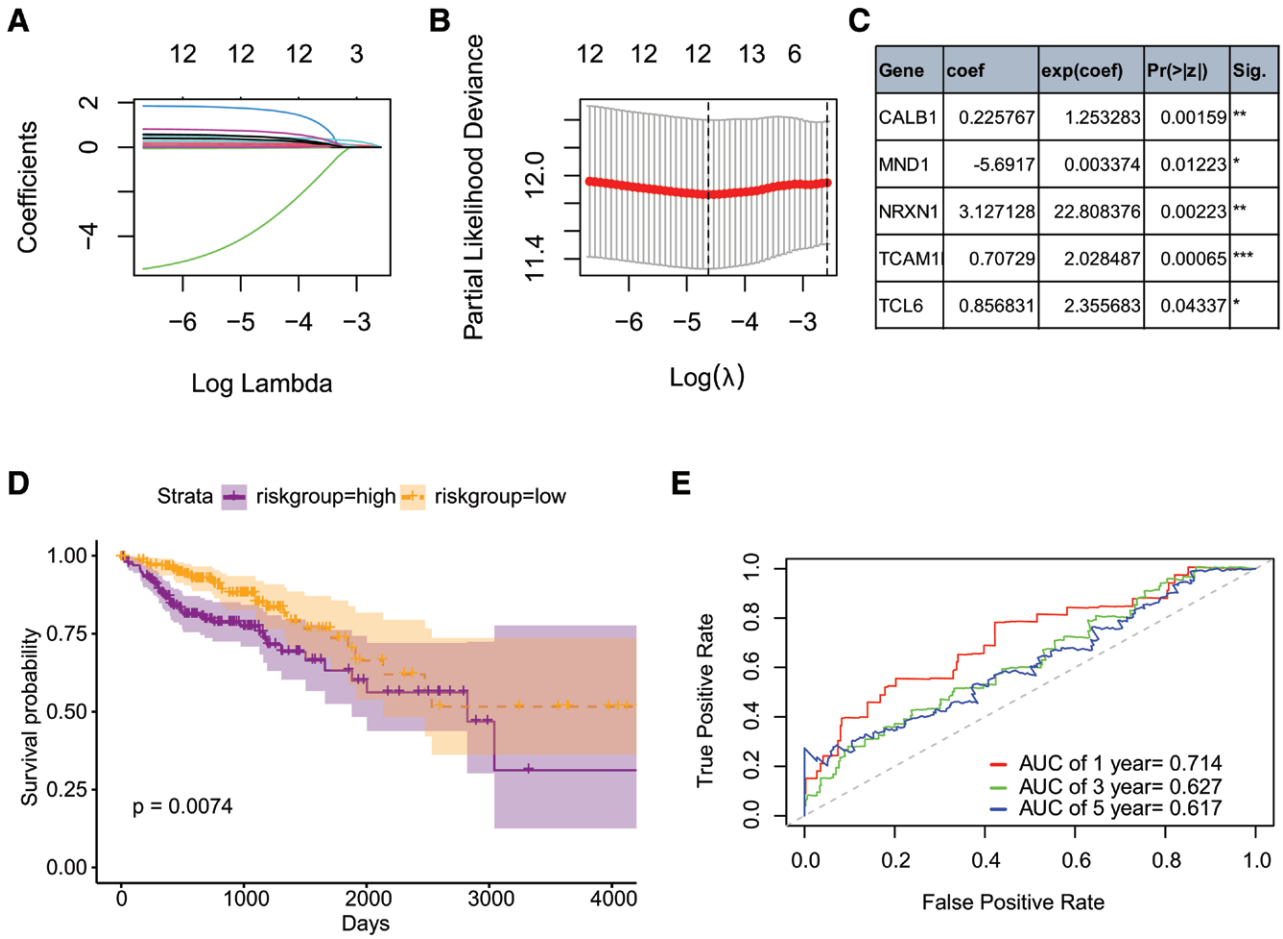


Figure 3. Mechanisms of FEN1-mediated drug resistance in colon cancer. (A) Differential drug sensitivity between High_FEN1 and Low_FEN1 groups in the TCGA-COAD cohort. (B) Volcano plot of differentially expressed genes between High_FEN1 and Low_FEN1 groups. (C) Top 5 activated and inhibited biological processes significantly enriched with differentially expressed genes. (D) Top 5 activated and inhibited KEGG pathways significantly enriched with differentially expressed genes. (E) Co-expression correlation heatmap between FEN1 and differentially expressed genes. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$. FEN1 = flap endonuclease 1, KEGG = Kyoto encyclopedia of genes and genomes, TCGA = The Cancer Genome Atlas.



Gene	coef	exp(coef)	Pr(>|z|)	Sig.
CALB1	0.225767	1.253283	0.00159	**
MND1	-5.6917	0.003374	0.01223	*
NRXN1	3.127128	22.808376	0.00223	**
TCAM1	0.70729	2.028487	0.00065	***
TCL6	0.856831	2.355683	0.04337	*

Figure 4. Colon cancer prognostic risk features derived from FEN1-associated differentially expressed genes. (A and B) Lasso Cox regression analysis identified 12 prognostic genes for colon cancer. (C) Multivariate Cox regression analysis identified 5 independent prognostic genes for colon cancer. (D) Survival curve showing the difference in overall survival between high-risk and low-risk groups of colon cancer patients. (E) Receiver operating characteristic curves of riskscore for predicting 1-year, 3-year, and 5-year overall survival in TCGA-COAD patients. FEN1 = flap endonuclease 1, TCGA = The Cancer Genome Atlas.

history and detailed pathological staging, appears promising for refining the prognostic stratification in colon cancer patients. MND1 serves as a prognostic factor in diverse malignancies,[35,36] and its involvement in a positive feedback loop with KLF6 and E2F1 has been observed, regulating the cell cycle and conferring drug resistance.[37] In small cell lung cancer, deletion of NRXN1 has been associated with the loss of antitumor activity mediated by NRXN1-mediated antibody-drug conjugate therapy.[38] Furthermore, decreased expression levels of the long noncoding RNA TCL6 have been linked to unfavorable prognosis in clear cell renal cell carcinoma[39] and pediatric B-cell acute lymphoblastic leukemia.[40] TCL6 is regulated by miR-155 and contributes to renal cancer progression and metastasis through its involvement in the Src-Akt-EMT network.[41] Nonetheless, the precise roles of these genes in colon cancer remain elusive, thereby underscoring their potential as novel therapeutic targets for intervention strategies.

Nevertheless, this study has several limitations. Firstly, the investigation into FEN1-mediated drug sensitivity in colon cancer and its underlying mechanisms was primarily based on computational analyses, lacking experimental validation. Secondly, the predictive efficacy of the FEN1-derived colon cancer risk features in different cohorts was not assessed. Finally, the biological functions of the genes within the risk features in colon cancer remain largely unexplored. Further comprehensive investigations using both experimental and clinical approaches are warranted to overcome these limitations and enhance our understanding in this field.

5. Conclusion

In conclusion, the findings of this study demonstrate a significant association between elevated FEN1 expression and colon cancer metastasis as well as drug sensitivity. The underlying mechanisms involve the spliceosome, DNA replication, cell cycle, and multiple signaling pathways. Furthermore, we examined the co-expression patterns and associated prognostic relevance of genes that interact with FEN1. Moreover, we constructed and assessed the multi-gene risk features in colon cancer. Further exploration is warranted to elucidate the co-expression mechanisms of these genes with FEN1 and their contributions to drug resistance and prognosis in colon cancer.

Author contributions

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Writing – review & editing: Chunhui Rao, Jingfei Tong.

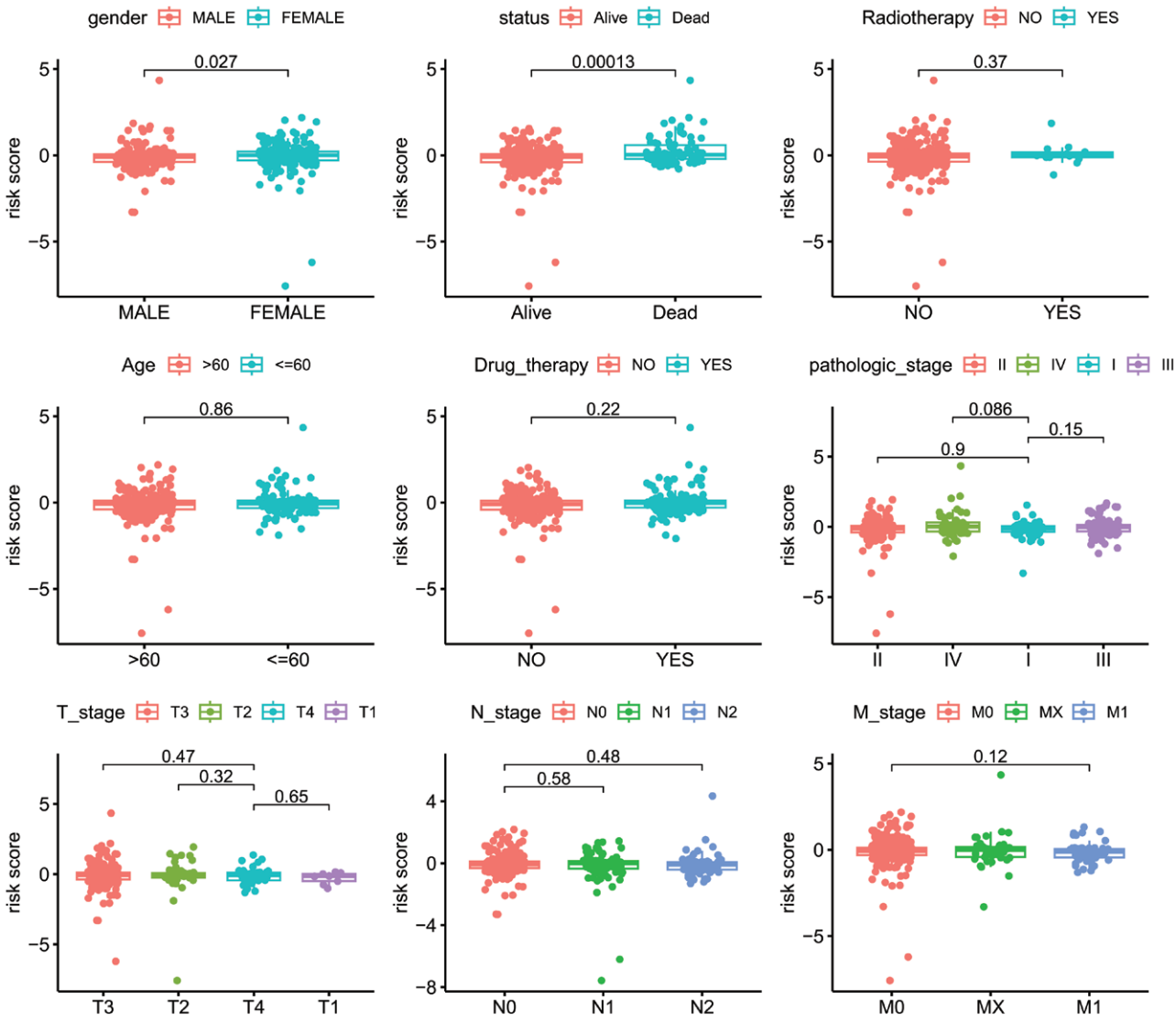

Figure 5. Comparison of riskscore differences among different clinical and pathological subgroups.

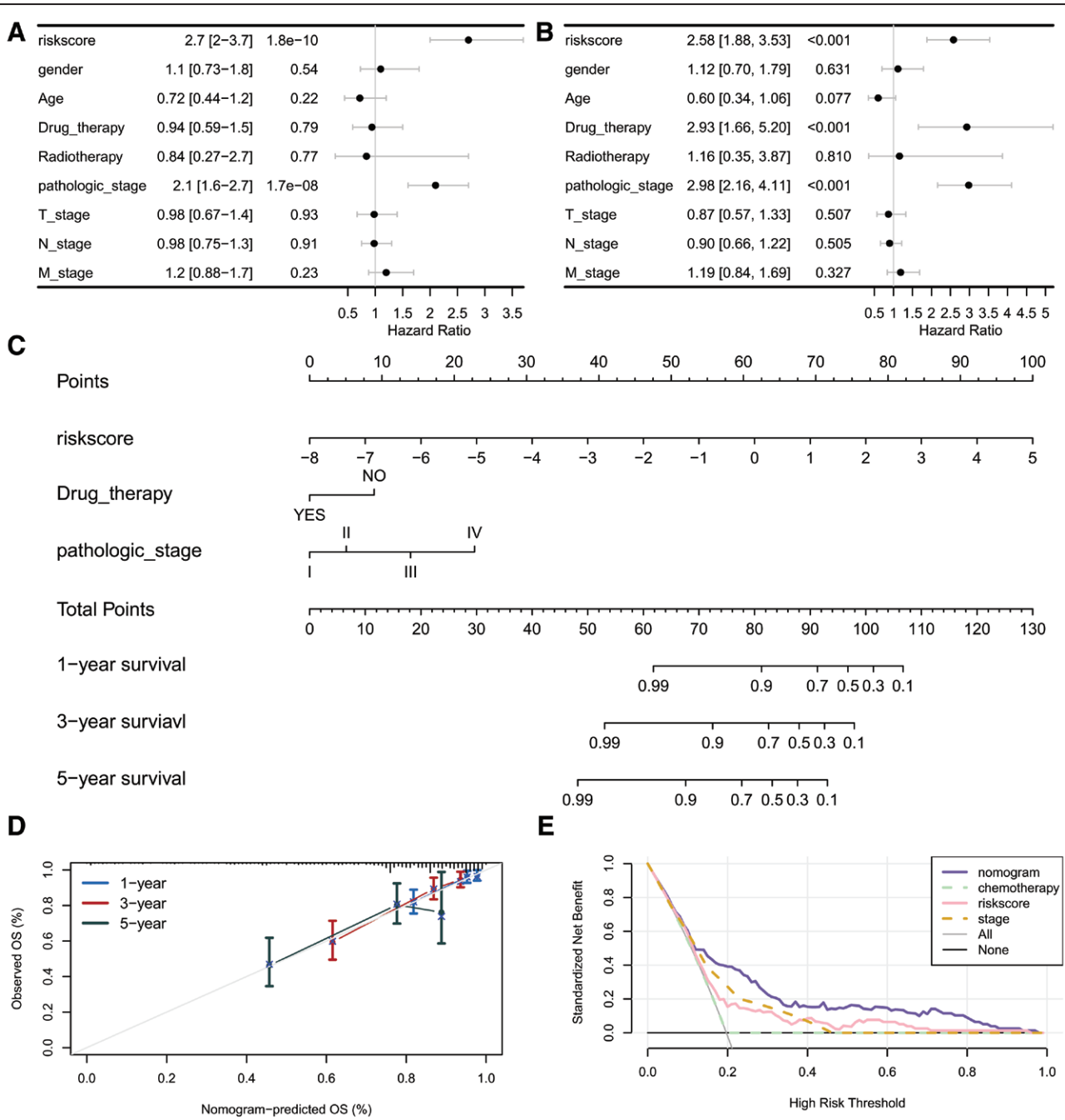

Figure 6. Construction and evaluation of a nomogram for colon cancer prognosis based on the TCGA-COAD cohort. Univariate (A) and multivariate (B) Cox analyses of key clinical characteristics and riskscore in colon cancer. (C) Nomogram model combining clinical-pathological factors and riskscore for predicting 1-year, 3-year, and 5-year survival rates. (D) Calibration curves for predicting 1-year, 3-year, and 5-year overall survival by combining key prognostic factors. (E) Decision curve analysis comparing the nomogram and independent prognostic factors for 1-year overall survival prediction. TCGA = The Cancer Genome Atlas.

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