Iran J Public Health, Vol. 54, No.4, Apr 2025, pp.762-774

Original Article

The Role of *TRPA1* as a Prognostic Marker in Colon Adenocarcinoma and its Correlation with Mutations and Immunity

*Xingxing Wu [1], Lifang Peng [1], Mingxu Zheng [2], Yuqing Mao [2], Heng Li [2], *Shaopeng Sun [2]*

1. *Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Shanghai, China*
2. *The First School of Clinical Medicine, Zhejiang Chinese Medical University, Hangzhou, China*

***Corresponding Author:** Email: sunshaopeng0903@qq.com

(Received 10 Mar 2024; accepted 18 May 2024)

Abstract

Background: This study aimed to investigate the prognostic value of TRP ion channel genes (TRPICGs) in colorectal adenocarcinoma (COAD) and explore its related mechanisms.

Methods: The COAD dataset was downloaded from the Cancer Genome Atlas (TCGA) database. The differential expression genes (DEGs) were screened between COAD and normal samples. The differentially expressed TRPICGs (DE-TRPICGs) were obtained via intersection of DEGs and 28 TRPICGs. The Kaplan-Meier (K-M) survival curve was used to screen DE-TRPICGs with survival differences as prognostic markers. Afterward, the correlation of prognostic marker with clinical, immune cell, copy number variation were explored. Finally, immunohistochemistry (IHC) was used to verify the expression of prognostic marker.

Results: Overall, 6003 DEGs were screened, and 6 DE-TRPICGs were obtained. Only *TRPA1* was identified as prognostic biomarker. Survival and clinical correlation analyses implied that *TRPA1* played an inhibitory role in colon adenocarcinoma pathogenesis and progression. Gene Set Enrichment Analysis (GSEA) indicated that *TRPA1* was associated with cell cycle and immune-related pathways. Immune infiltration analysis showed that *TRPA1* expression was significantly correlated with the infiltration of B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils and dendritic cells. Eventually, *TRPA1* expression was down-regulated at the protein level in COAD samples, which presented consistent results with expression in the database.

Conclusion: TRPA1 was identified in COAD as a prognostic marker associated with TRP ion channels, which provided a powerful reference value and a new direction for the diagnosis and treatment of COAD.

Keywords: Colon adenocarcinoma; Transient receptor potential channel; Prognosis; Infiltrating immune cells

Introduction

Colorectal cancer (CRC) occupies a prominent position among gastrointestinal cancers worldwide, with a global incidence increase of 4.2% annually. Colon adenocarcinoma (COAD), the most common colon cancer type, accounts for more than 80% of colorectal cancers (1, 2). Cancers at the early stage are often undetectable due to the absence of effective screening or highly sensitive and specific diagnostic indicators (3). Therefore, it is crucial to find new effective prognostic marker to facilitate early diagnosis (4).

Cancers develop through the progressive acquisition of somatic genetic alterations, such as somatic mutations and copy number variations (CNVs), that affect the function of key genes regulating cell growth and survival (5). In addition, the evolution of tumors is closely related to their microenvironment (6). Accumulating evidence indicates that specific tumor microenvironments (TME) exist in CRC and that the TME promotes tumor progression and metastasis by producing a variety of pro-cachectic factors (7).

The family of non-selective and selective cation channels, known as the transient receptor potential (TRP), are composed of TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPP (polycystin), TRPML (mucolipin) and TRPN (no mechanoreceptor potential C-NOMPC) channels and function as both cellular sensors and signal transducers. TRPs have been found to be involved in a variety of gastrointestinal functions (8), while changes in TRP levels are associated with various gastrointestinal disorders, such as gastroesophageal reflux disease (9), irritable bowel syndrome, and dyspepsia (10). Previous studies have shown that the expression of TRPM6 at the mRNA level is lower in colon cancer tissues than in normal tissues (11), and microarray analysis of 379 patients with colorectal cancer revealed that the high expression of TRPM4 protein is related to epithelial-mesenchymal transformation and colon tumor invasion (12). TRPs may play an important role in colon cancer.

Therefore, we aimed to explore the relationship between TRP ion channel genes and the prognosis of patients with COAD, investigate the possible mechanisms of *TRPA1* affecting the prognosis of patients, and provide a theoretical framework for further elucidating the molecular mechanism of COAD pathogenesis and progression.

Methods

Data source

The TCGA-COAD dataset was downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/), which includes 41 normal samples and 447 COAD samples. After deleting samples that cannot obtain clinical information, Overall, 390 COAD samples were selected for survival analysis. Twenty COAD paraffin samples and corresponding adjacent normal tissues were obtained from the sample bank of the First Affiliated Hospital of Zhejiang University of Traditional Chinese Medicine.

All experimental studies are conducted after ethical approval.

Identification of differentially expressed TRP ion channel genes

Differentially expressed genes (DEGs) between COAD and normal samples were analyzed according to the screening criteria of $|\log_2 \text{Fold Change (FC)}|>1$ and $P<0.05$ (13, 14). Differentially expressed TRP ion channel genes (DE-TRPICGs) were obtained by overlapping DEGs with 28 TRP ion channel genes (15-17).

Protein-protein interaction network construction

Protein-protein interaction (PPI) network was constructed to visualize the functional interactions among DEGs (18) based on the STRING website (https://string-db.org) with a confidence level of 0.15. The PPI graph was then embellished using the Cytoscape software (19).

Survival analysis

Patients were divided into DE-TRPICGs high expression group and low group, according to the median expression of DE-TRPICGs in COAD. The overall survival (OS) of patients with high and low COAD expression was analyzed, and a corresponding K-M curve was generated.

Gene set enrichment analysis (GSEA)

Gene Set Enrichment Analysis (GSEA) was conducted using the R packages 'clusterProfiler' and 'org.Hs.eg.db'. The correlation coefficient with all genes in the gene sets was calculated using the expression value of each gene as the phenotype data with the significant threshold of $|NES| >1$ and adjusted $P<0.05$.

Mutation and copy number variation analysis

Maftools is an R package that includes functions for identifying driver genes, pathways, signatures, enrichment, and association analysis (20). To investigate the somatic mutations in COAD samples, we downloaded somatic mutation data from the TCGA database and analyzed the data with the 'Maftools' R package. The mutation status of the specific gene was searched and obtained from the cBioPortal database (https://www.cbioportal.org/).

Analysis of infiltrating immune cells

The correlations between gene expression and infiltration of six types of immune cells were determined using a TIMER2.0 database (http://timer.cistrome.org/). The R package, 'MCPcounter', can quantify the absolute abundance of two stromal cells and eight immune cells using the transcriptome data and provide tumor infiltration levels, and the TIMER provides a comparison of tumors with somatic copy number changes for a given gene (21). Somatic copy number alterations (SCNAs) were defined by including deep deletion, arm-level deletion, diploid/normal, arm-level gain, and high amplification (22). Two-sided Wilcoxon rank-sum tests were performed to compare the infiltration levels for each SCNA category.

Immunohistochemistry

We randomly selected 10 COAD paraffin samples and corresponding adjacent normal tissues from the sample bank. The key genes protein levels in formalin-fixed paraffin-embedded COAD tissues and adjacent non-tumor tissues were detected by immunohistochemistry (IHC) using key genes antibodies (1:200, PROTEINTECH).

Statistical analysis

All statistical analyses were performed using R software. Data from different groups were compared using Wilcoxon tests. Statistical significance was considered to exist if the *P*-value was less than 0.05 unless otherwise specified.

Ethics approval

The research protocol of this study was approved by the Ethics Committee of The First Affiliated Hospital, Zhejiang Chinese Medicine University (number 2021-KL-201-01) and executed following all corresponding guidelines and regulations. All paraffin samples were obtained from the sample bank of the First Affiliated Hospital of Zhejiang University of Traditional Chinese Medicine and the Ethics Committee approved the application report for exemption from informed consent.

Results

In all 6 DE-TRPICGs were screened in COAD

We first screened DEGs between COAD and normal samples in the TCGA-COAD cohort and detected a total of 6003 DEGs, including 3257 up-regulated genes and 2746 down-regulated genes (Fig.1A, Supplementary Table 1) (Supplementary tables are not showed). By intersecting with 28 TRP ion channel genes, we found six differentially expressed TRP ion channel genes (DE-TRPICGs), namely *TRPM6*(log_2FC=-2.79, P<0.05), *TRPV3*(log_2FC=-0.74, P<0.05), *TRPM4*(log_2FC=-1.02, P<0.05), *MCOLN2* (log_2FC=-1.24, P<0.05), *TRPA1*(log_2FC=-0.91, P<0.05), and *TRPM2*(log_2FC=-1.01, P<0.05) (Fig. 1B). Among them, *TRPM2* was up-regulated, while *TRPM6*, *TRPV3*, *TRPM4*, *MCOLN2*, and *TRPA1* were down-regulated in COAD samples compared to normal tissues (Fig. 1C). To understand further the interaction of the proteins encoded by these six genes, we created an interaction network of these six proteins using STRING and displayed it in Fig. 1D.

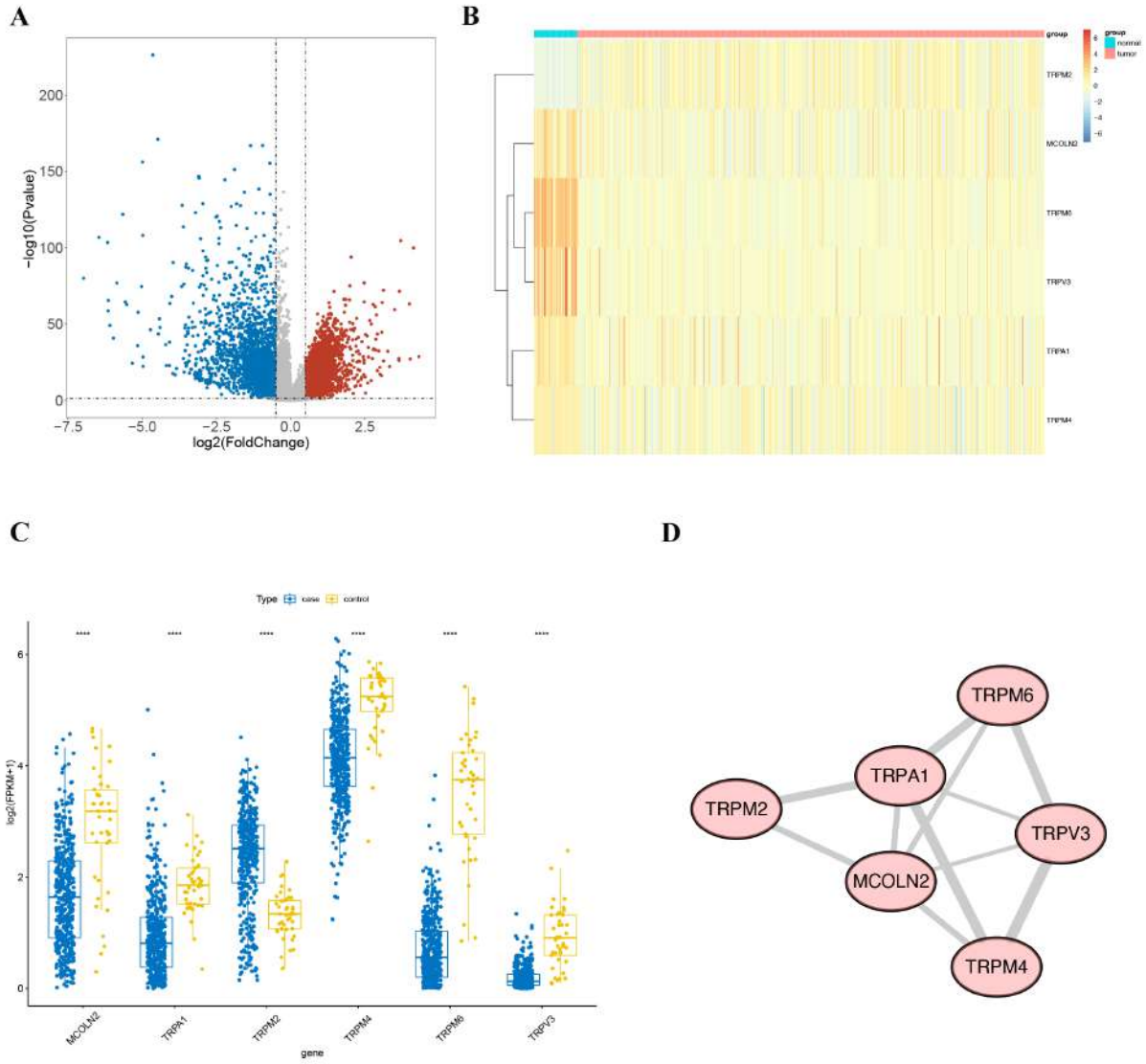

Fig. 1: Differentially expressed TRP ion channel genes (DE-TRPICGs) in colon adenocarcinomas (COAD) samples. A. Volcano plot showing differential expressed genes in COAD. B. Heatmap indicating the DE-TRPICGs expression in COAD. C. The box plot showing the expression of the six TRPICGs. D. Protein-protein interaction (PPI) network of the six TRPICGs

TRPA1 was identified as a prognostic marker and its expression correlated with tumor stage, T stage and N stage

To further determine whether the six DE-TRPICGs affect the survival of COAD patients, we performed the corresponding survival analysis. Only *TRPA1* was correlated to the survival of COAD patients (P<0.05) and served as a prognostic marker (Fig. 2A-F). Moreover, the survival rate of COAD patients in the high *TRPA1* expression group was significantly higher than that of COAD patients in the low *TRPA1* expression group (P<0.05) (Fig. 2B). We compared *TRPA1* expression in subgroups with different clinical characteristics (Fig. 2G-J). *TRPA1* expression was associated with tumor stage, T-stage, and N-stage, but not M-stage (Fig. 2H). The expression of *TRPA1* was significantly lower in stage III-IV

samples than in stage I-II samples (P<0.05) (Fig. 2J). Meanwhile, the expression of *TRPA1* was significantly lower in T3-T4 stage samples than in T1-T2 stage samples (P<0.05) (Fig. 2G). The expression of *TRPA1* was significantly reduced in N1-N2 stage samples compared with N0 stage samples (P<0.05) (Fig. 2I). *TRPA1* was downregulated in stages with higher malignancy and poorer prognosis, implying that *TRPA1* played an inhibitory role in the pathogenesis and progression of COAD.

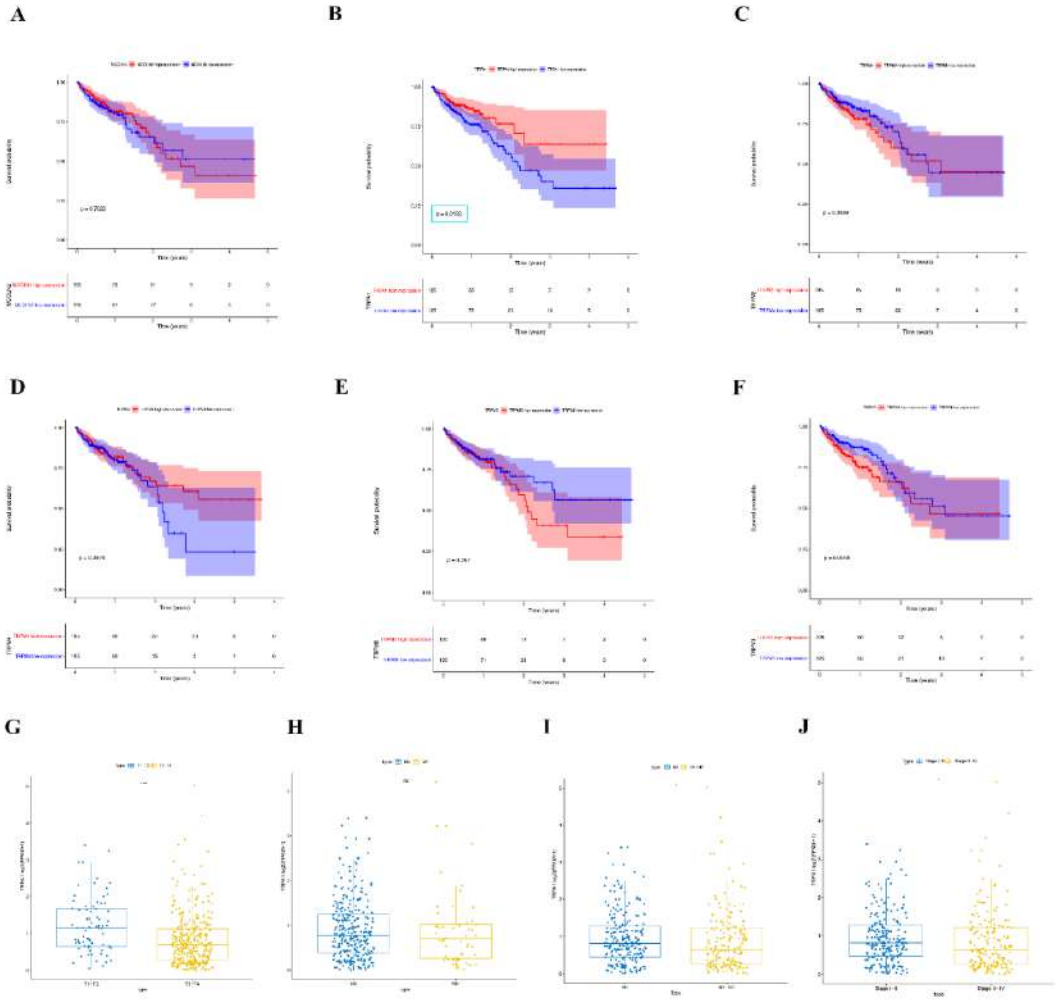

Fig. 2: Association of TRPA1 levels in COAD samples with the survival of COAD patients. A-F. Kaplan-Meier curve of patients with high and low expression of the six DE-TRPICGs. TRPA1 expression in T stage (G), N stage (H), and M stage (I) and COAD stage (J)

Functional enrichment analysis of high and low expression of TRPA1

To further explore the functional role of *TRPA1* in COAD progression, we took *TRPA1* as the target gene and calculated the correlation coefficient between the expression of all other genes and *TRPA1*. Using the correlation coefficient as the ranking standard, we performed GSEA enrichment analysis. The results are listed in Supplementary Table 2. The gene ontology (GO) annotation showed that low *TRPA1* expression was associated with 'catabolic process', 'amino acid metabolic process', 'protein complex disassembly' in the biological processes (BP), 'cyto-

solic large ribosomal subunit', 'organellar ribosome'in cell component (CC), and 'antigen binding', 'catalytic activity acting on a tRNA'in molecular function (MF). The Fig. 3A-C revealed the top 10 gene ontology (GO) annotation. Similarly, KEGG pathway enrichment showed that low TRPA1 expression was related to 'cell cycle' and 'DNA replication', while high TRPA1 expression was associated with 'calcium signaling pathway' and 'glycan biosynthesis'. TRPA1 played an important role in the key biological processes of COAD (Fig. 3D).

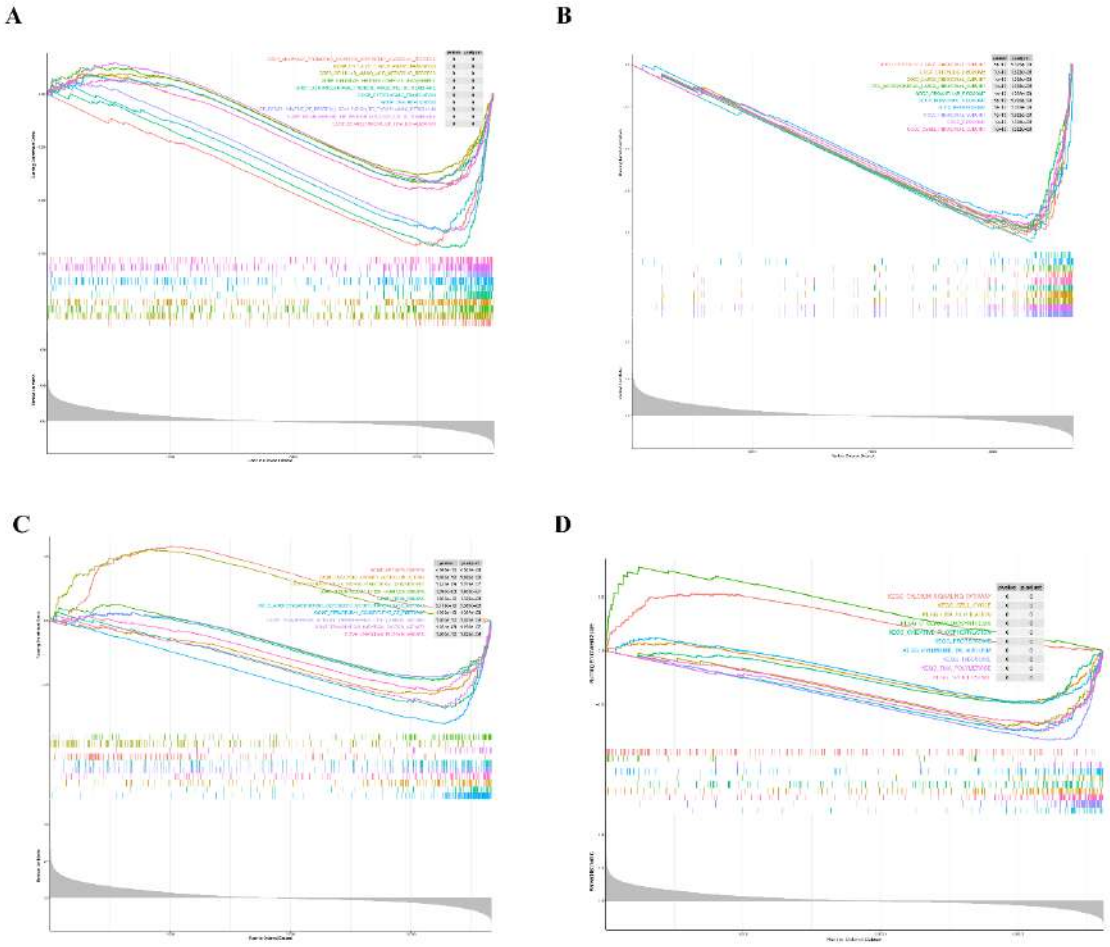

Fig. 3: Top ten GO items (A-C) and KEGG pathways (D) enriched by COAD in GSEA analysis

Investigation of mutational of TRPA1 in COAD samples

To gain further insight into somatic mutations in COAD samples in the TCGA-COAD cohort, relevant data were extracted and proceeded. Fig. 4A shows the overall mutation information in COAD samples, including variation classification, variation type, single nucleotide polymorphisms (SNVs), and top ten mutated genes. Notably, missense mutations were the predominant mutation type. We also analyzed the somatic mutations in *TRPA1* and found that only 7% of COAD samples had *TRPA1* mutations, including amplification, missense, splicing, and truncating mutations (Fig. 4C). We then compared the difference in survival between patients with and without *TRPA1* mutations. However, the difference was not significant (Fig. 4B).

We also performed correlation analysis and identified the top ten genes that were positively and

negatively associated with *TRPA1* expression (Supplementary Table 3). For the top 20 genes, we performed somatic mutation analysis and noted that 16 genes had mutations (Fig. 4D). Among them, the mutation rates of *ADGRL3* and *SALL1* were higher than 20%, with the majority being missense mutations (Fig. 4D). The relationship of these 16 genes mutations was shown in Fig.4E. Each square represents the interaction relationship between the two genes, and the darker the color, the tighter the interaction relationship (green indicates co-occurrence, red indicates mutually exclusive). Mutations of *TRPA1*-related genes may play a crucial role in promoting colon cancer.

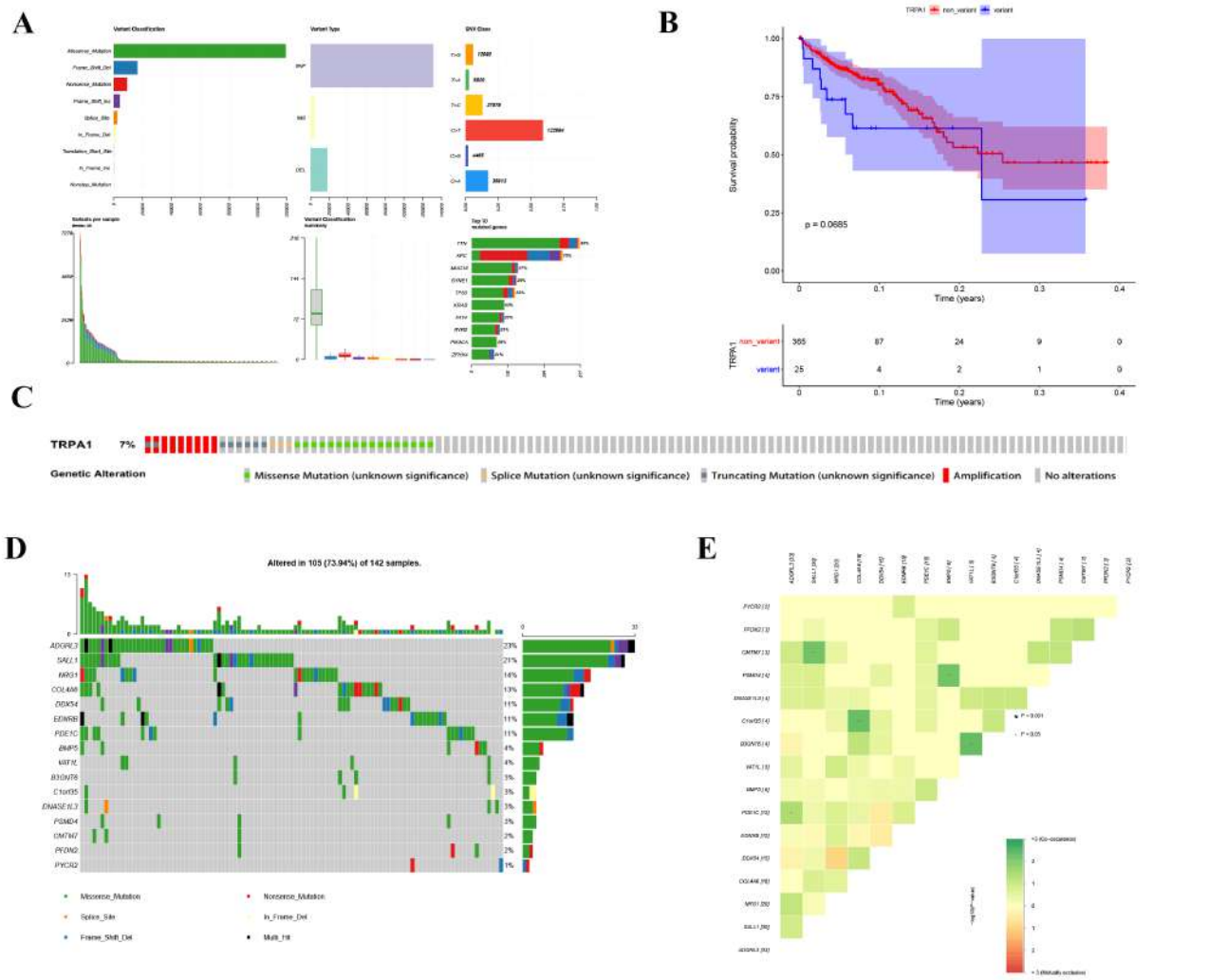

Fig. 4: TRPA1 and mutations in COAD samples. A. Summary of mutation information in COAD samples. B. K-M survival curves of patients with mutated and non-mutated TRPA1. C. Mutations of TRPA1 gene in COAD samples. D. Waterfall plot to display the mutations in 16 TRPA1-related genes in COAD samples. E. The correlation analysis of 16 TRPA1-related mutant genes

The expression level of TRPA1 was related to the CNV of Gain and Diploid

We analyzed the type of CNVs, including shallow deletion, diploid, gain, and amplification, in *TRPA1* and their effects on *TRPA1* expression utilizing the cBioPortal database (23). Most CNV types were distributed in diploid and gain. In addition, patients with Gain in *TRPA1* showed significantly higher *TRPA1* expression than patients with Diploid in *TRPA1* (Fig. 5). Therefore, the expression level of *TRPA1* may be related to CNV of Gain and Diploid, but the causal relationship is still unknown.

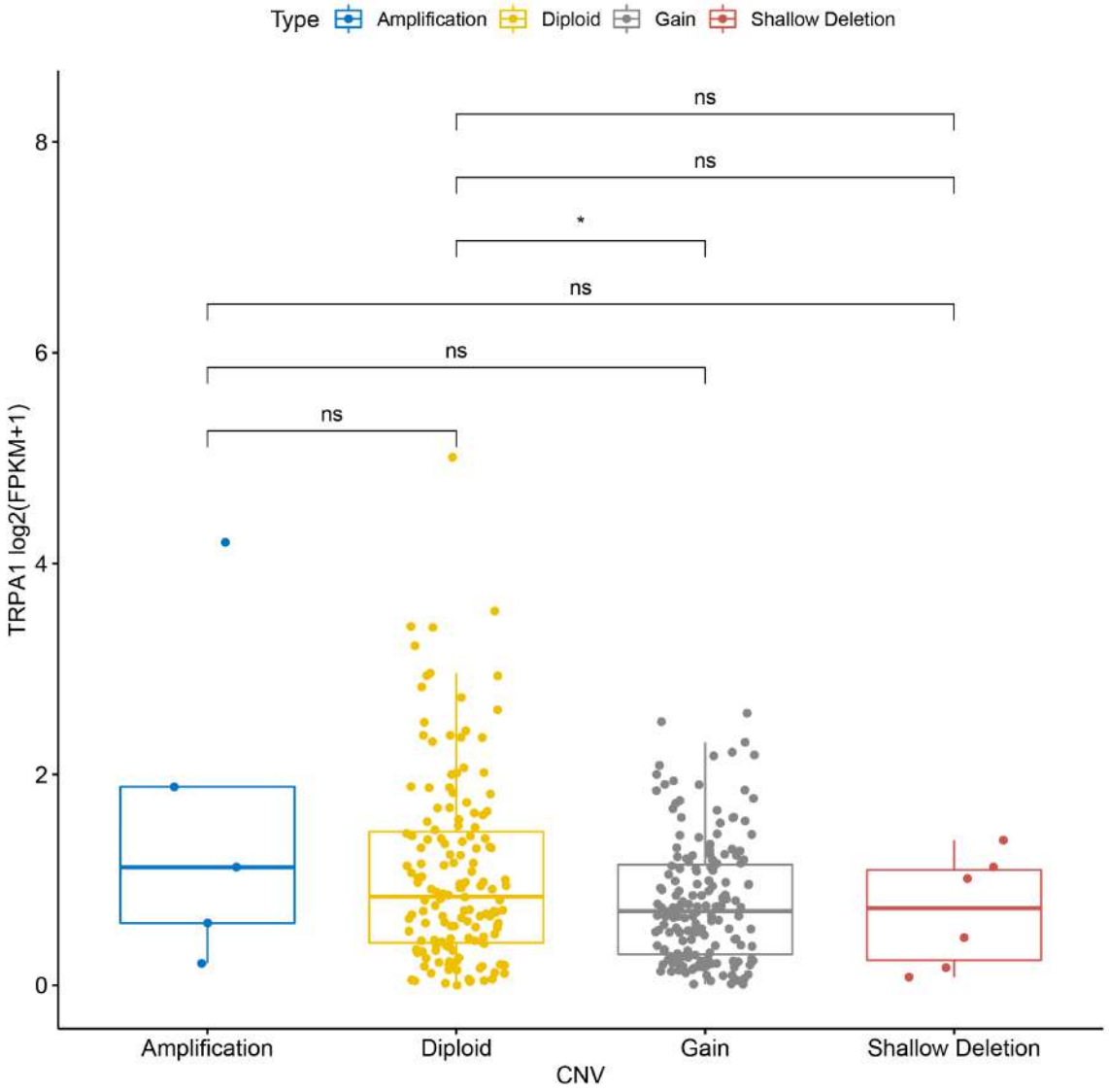

Fig. 5: The correlation between TRPA1 gene expression and its copy number variation types

Immune cell infiltration and CNV were correlated with the expression of TRPA1

TRPA1 expression was related to immune-related pathways (Supplementary Table 2). Hence, we further analyzed the correlation between *TRPA1* expression and infiltration of six types of immune cells using the TIMER database. TRPA1 expression was significantly correlated with the

infiltration of five types of immune cells, including B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, and dendritic cells (P<0.05) (Fig. 6A). Besides, we divided COAD samples into high and low *TRPA1* expression groups and compared the difference in immune infiltration between the two groups. The infiltration of most immune cells were related to *TRPA1* expression (Fig. 6B). We further explored the relationship between immune cell infiltration and *TRPA1* CNVs and analyzed the role of *TRPA1* CNVs in immune cell infiltration. Fig. 6C shows the distribution of immune subsets at various copy number levels in COAD samples. Arm-level gain significantly affected the infiltration level of B cells, arm-level deletion and arm-level gain significantly affected the infiltration level of $CD8^+$ T cells, and arm-level gain and amplification significantly affected the infiltration level of dendritic cells.

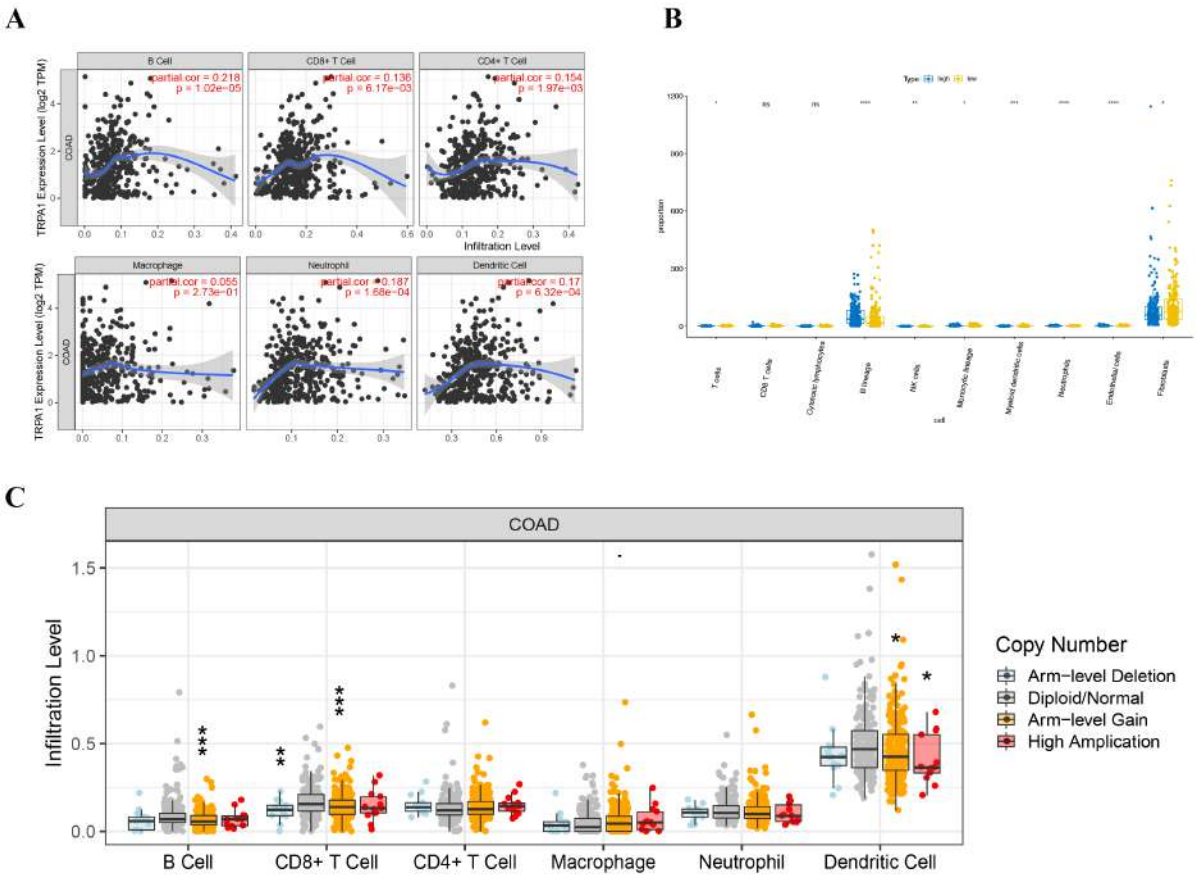

Fig. 6: TRPA1 was related to immune microenvironment. A. The correlation between TRPA1 gene expression and six immune cells. B. Difference of immune infiltration between the TRPA1 high and low expression group. C. Effect of the Genetic Alterations of TRPA1 on the immune cell infiltration

TRPA1 expression was down-regulated in COAD samples

We randomly selected ten pairs of COAD samples and corresponding adjacent normal samples from the sample bank for further verification. TRPA1 was downregulated in COAD samples compared with normal tissues in protein level (Fig. 7), which is in good agreement with the previous gene analysis (Fig. 1C).

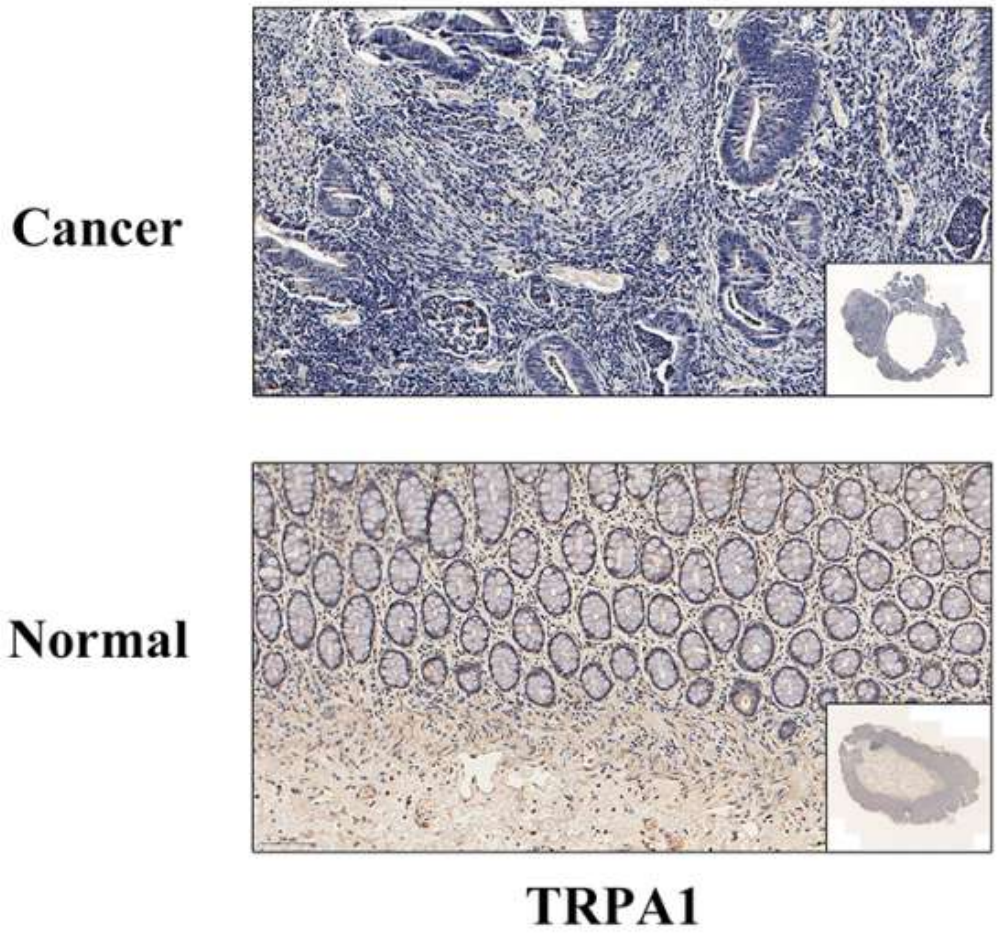

Fig. 7: TRPA1 Expression in colon cancer and normal samples

Discussion

As one of the most prevalent cancer types, cancers of the digestive system are often diagnosed at an advanced stage, resulting in a poor prognosis (24). The relationship between TRP ion channel levels and gastrointestinal cancers has become the focus of many researchers (25). In this study, we downloaded the sequencing data, clinical data, mutations data, CNVs data, and immune data of COAD patients from the TCGA database and analyzed the genetic signature and prognostic value of *TRPA1* in COAD patients.

In this study, six TRP ion channel genes were differentially expressed, and the levels of proteins they encoded were related to each other. However, in the subsequent survival analysis, only the expression of *TRPA1* was found to be associated with the prognosis of COAD patients. Besides, *TRPA1* was downregulated in COAD patients, and a higher *TRPA1* expression was associated with longer survival of COAD patients. Additionally, *TRPA1* expression was low in cancer tissues compared to adjacent normal tissues using clinical samples. Further, we collated patients' pathological data, including tumor (T), node (N), and metastasis (M), and divided them into high and low *TRPA1* expression groups. The expression of *TRPA1* was low in the stage III-IV group, N1-N2 group and T3-T4 group comparing stage I-II , N0 and T1-T2, but there was no significant difference in the stage M0-M1. *TRPA1* might be a strong prognostic marker for COAD. Thus, we performed a single-gene GSEA

analysis of and the results showed that the low expression of *TRPA1* is related to "cell cycle" and its high expression is related to "calcium signaling pathway". The abnormality of cell cycle progression is one of the basic mechanisms of tumor occurrence. Treatment targeting the components of cell cycle mechanisms can not only inhibit cancer cell division, but also reverse cancer metabolism, restore cancer immune monitoring, and so on (26). Calcium signaling is a crucial second messenger in intracellular and intercellular signaling pathways, contributing to the progression of cancer(27). It can intervene in the development of colon cancer by regulating cell cycle (28-30). Upregulation of RFC2 and downregulation of miR-744 was observed in CRC tissues (30). RFC2 knockdown promotes cell cycle arrest in G1 phase by downregulating cyclin E2 (CCNE2) both in vivo and in vitro, thereby inhibiting CRC cell proliferation. A recent study can prevent the migration and invasion of CRC by activating intracellular calcium signals. TRPM4 activates calpain mediated FAK cleavage through intracellular Ca2+signaling and prevents CRC migration and invasion by regulating the PI3K/Akt/mTOR signaling cascade (31). Similarly, we found that TRPA1 may be related to colon cancer progression through cell cycle and ion channel mechanisms.

Cancer is generally believed to be a clonal proliferative disease originating from somatic mutations to obtain genomic instability (32). Our results showed that 7% of COAD samples harbored mutations in *TRPA1*, including amplification, missense, splicing, and truncation mutations. No significant difference in survival was found between the *TRPA1* mutation and non-mutation groups. However, among the 20 genes highly related to *TRPA1*, 16 have mutations, and most of their expression are highly correlated. Therefore, we suspect *TRPA1* may play a bridge role in other gene mutations. Genes targeted by CNVs play a central role in tumorigenesis therapy (33, 34), and accurate detection of CNVs is essential to cancer genome profiling, which can increase cancer prognosis and improve treatment decisions. We further analyzed the CNV types of *TRPA1* and their relationship with *TRPA1* expression. The results showed that CNV types of COAD patients mainly existed in Gain and Diploid, and *TRPA1* expression was generally low, which was consistent with the previous results showing that *TRPA1* was downregulated in tumor patients. *TRPA1* expression in patients with Gain-type CNVs was lower than that in patients with Diploid-type CNVs, suggesting that patients with Gain-type CNVs may have a worse prognosis. However, more data are needed to verify our conjecture.

Expression of *TRPA1* was positively correlated with the infiltration of five types of immune cells, including $CD8^+$ T cells, a typical anti-tumor immune cells that can recognize tumor cells through antigens and produce cytotoxic molecules to kill them (35). Neutrophils can activate and regulate innate and adaptive immune cells as an effector. Therefore, neutrophils play a crucial role in protecting the body against intracellular pathogens, autoimmunity, chronic inflammation, and cancers (36, 37). B cells in our bodies are considered the primary effector cells of humoral immunity, which suppress tumor progression by secreting immunoglobulins, causing T cells to respond, and directly killing cancer cells (38). *TRPA1* is involved in the COAD immune microenvironment. Recently, various studies have demonstrated the significant impact of CNVs on immune responses (39-41). Our study also explored the correlation between CNVs of *TRPA1* and immune cell infiltration in COAD. Some CNVs of *TRPA1* could inhibit immune cell infiltration.

Our study has a drawback. Some results in this study were inferred from bioinformatics without being experimentally verified. Future experiments are needed to validate further our results.

Conclusion

TRPA1 plays a bridge role in colon cancer gene mutation. It might be a prognostic marker for COAD. In addition, *TRPA1* expression is closely related to immune infiltration and CNVs, which

may be the reason for the better prognosis of COAD patients with higher *TRPA1* expression.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Data availability statement

Supplementary tables might be requested by respected readers from the corresponding author for reasonable application. Our results were generated using some of the basic data from TCGA database (https://portal.gdc.cancer.gov).

Funding

No funding.

Conflicts of Interest

The authors declare no competing interests.

References

1. Li M, Gu J (2005). Changing patterns of colorectal cancer in China over a period of 20 years. *World J Gastroenterol,* 11 (30):4685-8.
2. Biller LH, Schrag D (2021). Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. *JAMA,* 325 (7):669-685.
3. Garborg K, Holme Ø, Løberg M, et al (2013). Current status of screening for colorectal cancer. *Ann Oncol,* 24 (8):1963-72.
4. Das V, Kalita J, Pal M (2017). Predictive and prognostic biomarkers in colorectal cancer: A systematic review of recent advances and challenges. *Biomed Pharmacother,* 87:8-19.
5. Stratton MR, Campbell PJ, Futreal PA (2009). The cancer genome. *Nature,* 458 (7239):719-24.
6. Angell HK, Bruni D, Barrett JC, et al (2020). The Immunoscore: Colon Cancer and Beyond. *Clin Cancer Res,* 26 (2):332-339.
7. Kasprzak A (2021). The Role of Tumor Microenvironment Cells in Colorectal Cancer (CRC) Cachexia. *Int J Mol Sci,* 22 (4): 1565.
8. Alaimo A, Rubert J (2019). The Pivotal Role of TRP Channels in Homeostasis and Diseases throughout the Gastrointestinal Tract. *Int J Mol Sci,* 20 (21): 5277.
9. Matsumoto K, Suenaga M, Mizutani Y, et al (2021). Role of transient receptor potential vanilloid subtype 2 in lower oesophageal sphincter in rat acid reflux oesophagitis. *J Pharmacol Sci,* 146 (3):125-135.
10. Akbar A, Yiangou Y, Facer P, et al (2008). Increased capsaicin receptor TRPV1-expressing sensory fibres in irritable bowel syndrome and their correlation with abdominal pain. *Gut,* 57 (7):923-9.
11. Ibrahim S, Dakik H, Vandier C, et al (2019). Expression Profiling of Calcium Channels and Calcium-Activated Potassium Channels in Colorectal Cancer. *Cancers (Basel),* 11 (4):561.
12. Kappel S, Stokłosa P, Hauert B, et al (2019). TRPM4 is highly expressed in human colorectal tumor buds and contributes to proliferation, cell cycle, and invasion of colorectal cancer cells. *Mol Oncol,* 13 (11):2393-2405.
13. Ritchie ME, Phipson B, Wu D, et al (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res,* 43 (7):e47.
14. Phipson B, Lee S, Majewski IJ, et al (2016). Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. *Ann Appl Stat,* 10 (2):946-963.
15. Shapovalov G, Ritaine A, Skryma R, et al (2016). Role of TRP ion channels in cancer and tumorigenesis. *Semin Immunopathol,* 38 (3):357-69.
16. Venkatachalam K, Montell C (2007). TRP channels. *Annu Rev Biochem,* 76:387-417.
17. Ramsey IS, Delling M, Clapham DE (2006). An introduction to TRP channels. *Annu Rev Physiol,* 68:619-47.
18. Hao T, Peng W, Wang Q, et al (2016). Reconstruction and Application of Protein-Protein Interaction Network. *Int J Mol Sci,* 17 (6):907.

19. Li J, Li Z, Zhao S, et al (2020). Identification key genes, key miRNAs and key transcription factors of lung adenocarcinoma. *J Thorac Dis,* 12 (5):1917-1933.
20. Mayakonda A, Lin DC, Assenov Y, et al (2018). Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome Res,* 28 (11):1747-1756.
21. Li T, Fu J, Zeng Z, et al (2020). TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res,* 48 (W1):W509-W514.
22. Mermel CH, Schumacher SE, Hill B, et al(2011). GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biol,* 12 (4):R41.
23. Unberath P, Knell C, Prokosch H-U, et al(2019). Developing New Analysis Functions for a Translational Research Platform: Extending the cBioPortal for Cancer Genomics. *Stud Health Technol Inform,* 258:46-50.
24. Bray F, Ferlay J, Soerjomataram I, et al (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin,* 68 (6):394-424.
25. Stoklosa P, Borgström A, Kappel S, et al (2020). TRP Channels in Digestive Tract Cancers. *Int J Mol Sci,* 21 (5): 1877.
26. Liu J, Peng Y, Wei W (2022). Cell cycle on the crossroad of tumorigenesis and cancer therapy. *Trends Cell Biol,* 32 (1):30-44.
27. Sadras F, Monteith GR, Roberts-Thomson SJ (2021). An Emerging Role for Calcium Signaling in Cancer-Associated Fibroblasts. *Int J Mol Sci,* 22 (21):11366.
28. Han YH, Mun JG, Jeon HD, et al (2019). Betulin Inhibits Lung Metastasis by Inducing Cell Cycle Arrest, Autophagy, and Apoptosis of Metastatic Colorectal Cancer Cells. *Nutrients,* 12 (1):66.
29. Chen Y, Liu F, Chen X, et al (2024). microRNA-622 upregulates cell cycle process by targeting FOLR2 to promote CRC proliferation. *BMC Cancer,* 24 (1):26.
30. Hu T, Shen H, Li J, et al (2020). RFC2, a direct target of miR-744, modulates the cell cycle and promotes the proliferation of CRC cells. *J Cell Physiol,* 235 (11):8319-8333.
31. Wang C, Chen J, Kuang Y, et al (2022). A novel methylated cation channel TRPM4 inhibited colorectal cancer metastasis through Ca(2+)/Calpain-mediated proteolysis of FAK and suppression of PI3K/Akt/mTOR signaling pathway. *Int J Biol Sci,* 18 (14):5575-5590.
32. Tomasetti C, Li L, Vogelstein B (2017). Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. *Science,* 355 (6331):1330-1334.
33. Santarius T, Shipley J, Brewer D, et al (2010). A census of amplified and overexpressed human cancer genes. *Nat Rev Cancer,* 10 (1):59-64.
34. Liu S, Shao F, Wang Y, et al(2024). COX6C expression driven by copy amplification of 8q22.2 regulates cell proliferation via mediation of mitosis by ROS-AMPK signaling in lung adenocarcinoma. *Cell Death Dis,* 15 (1):74.
35. Zhang L, Yu X, Zheng L, et al (2018). Lineage tracking reveals dynamic relationships of T cells in colorectal cancer. *Nature,* 564 (7735):268-272.
36. Mantovani A, Cassatella MA, Costantini C, et al (2011). Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol,* 11 (8):519-31.
37. Quail DF, Amulic B, Aziz M, et al (2022). Neutrophil phenotypes and functions in cancer: A consensus statement. *J Exp Med,* 219 (6): e20220011.
38. Tokunaga R, Naseem M, Lo JH, et al (2019). B cell and B cell-related pathways for novel cancer treatments. *Cancer Treat Rev,* 73:10-19.
39. Fanciulli M, Petretto E, Aitman TJ (2010). Gene copy number variation and common human disease. *Clin Genet,* 77 (3):201-13.
40. Wang N, Liu D (2021). Identification and Validation a Necroptosis-related Prognostic Signature and Associated Regulatory Axis in Stomach Adenocarcinoma. *Onco Targets Ther,* 14:5373-5383.
41. Saitou M, Gokcumen O (2020). An Evolutionary Perspective on the Impact of Genomic Copy Number Variation on Human Health. *J Mol Evol,* 88 (1):104-119.