World Journal of Surgery and Surgical Research

Research Article
Published: 13 May, 2023

Analysis of the Significance of DNASE1L3 in Immune Infiltration of Colon Adenocarcinoma

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Abstract

Background: Several studies have shown the crucial role of DNASE1L3 in regulating immune function in various diseases including Systemic Lupus Erythematosus (SLE) and cancers. However, the function and expression of DNASE1L3 in Colon Adenocarcinoma (COAD) remain obscure. The aim of this study was to explore the immune function of DNASE1L3 in COAD through a comprehensive bioinformatic analysis.

Objective: To study DNASE1L3 expression in colorectal cancer through multiple databases. Use bioinformatics analysis to learn about the influence of this gene on clinical prognosis and immune function in colorectal cancer, and to explore its potential biological function.

Methods: We obtained transcriptome data of COAD and normal samples from The Cancer Genome Atlas (TCGA) and identified Differentially Expressed (DE) mRNAs. The difference mRNA between COAD and normal samples and the difference mRNA between stage I and stage IV were respectively excavated, and then the intersection was taken. DNASE1L3 was determined the significant DE mRNA. Further, we obtained the different expressions of DNASE1L3 integrates the normal tissue data in the TCGA tumor tissue data to analyze the expression differences of 20 tumors. DNASE1L3 was further subjected to an analysis of expression in a different stage of COAD. We evaluated the influence of DNASE1L3 on clinical prognosis using Gene Expression Profiling Interactive Analysis (GEPIA) in COAD patients. To demonstrate the relationship between immune function and DNASE1L3, we investigated whether DNASE1L3 expression is related to the level of immune infiltration in COAD. We separately counted the number of neoantigens in COAD and analyzed the relationship between DNASE1L3 expression and the number of antigens. We analyzed the correlation between DNASE1L3 expression and MSI (Microsatellite Instability), using Spearman's rank correlation coefficient. In addition, we analysis the enrichment function of DNASE1L3. Finally, we performed immunohistochemical analysis on tissue microarray of colon adenocarcinoma.

Results: In this study, we observed significantly down-regulated expression of DNASE1L3 in many different cancers including COAD, which also correlated with grade. Low expression of DNASE1L3 was significantly correlated with poorer Overall Survival (OS) in COAD (OS HR=0.4, P=0.032). Low expression of DNASE1L3 was found with a poor prognosis. DNASE1L3 expression was positively correlated with infiltrating levels of CD4+ T and CD8+ T cells, B cells, macrophages, neutrophils and Dendritic Cells (DCs) in COAD. DNASE1L3 expression showed strong correlations with diverse immune marker sets in COAD. Enrichment functional analysis revealed that DNASE1L3 was associated with immunoglobulin complexes, antibacterial humoral responses, and humoral immune responses.

Conclusion: These findings suggest that DNASE1L3, which functions as a tumor suppressor gene in COAD, might be a potential therapeutic target. It is correlated with prognosis and immune infiltrating levels, including those of CD4+ T and CD8+ T cells, B cells, macrophages, neutrophils and Dendritic Cells (DCs) in COAD patients. These findings suggest that DNASE1L3 can be used as a prognostic biomarker for determining prognosis and immune infiltration in COAD. These findings suggest that DNASE1L3 can be used as a prognostic biomarker for determining prognosis and immune infiltration in COAD.

Keywords: Colonic adenocarcinoma; Colorectal cancer; Bioinformatic analysis; DNASE1L3; TCGA; MSI

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Received Date: *24 Apr 2023*
Accepted Date: *08 May 2023*
Published Date: *13 May 2023*

Citation:
Chen J, Zhu J, Sun P, Wu W, Liu Z. Analysis of the Significance of DNASE1L3 in Immune Infiltration of Colon Adenocarcinoma. World J Surg Surgical Res. 2023; 6: 1465.

Abbreviations

COAD: Colonic Adenocarcinoma; CRC: Colorectal Cancer; TCGA: The Cancer Genome Atlas; KEGG: Kyoto Encyclopedia of Gene and Genome; GO: Gene Ontology; TMA: Tissue Microarray; DEGs: Differentially Expressed Genes; MSI: Microsatellite Instability; FDR: False Discovery Rate; PPI: Protein-Protein Interaction; OS: Overall Survival; mRNA: messenger RNA; IHC: Immunohistochemical Staining; SLE: Systemic Lupus Erythematosus; DC: Dendritic Cells; IARC: International Agency for Research on Cancer; WHO: World Health Organization; HDI: Human Development Index; NHGRI: National Human Genome Research Institute; NCI: National Cancer Institute; TSNAD: Tumor Specific Neoantigen Detector; MHC: Major Histocompatibility Complex; TIMER: Tumor Immune Estimation Resource; ROC: Receiver Operating Characteristic curve; HCC: Hepatocellular Carcinoma

Introduction

Cancer statistics in 2020 show that the number of new cases and deaths of digestive system tumors ranks first, while the number of new cases and deaths of Colon Cancer (CC) ranks first in the digestive system, and the number of new cases is far higher than other digestive system tumors [1]. In China, colorectal cancer incidence and mortality are also at the forefront, a serious threat to public health and society a heavy financial burden [2]. The pathogenesis of CC is still unclear, and it is generally believed to be related to a variety of factors such as genetics, diet, inflammation, immunity, and intestinal microecology [3]. Colon Adenocarcinoma (COAD) is the most common form of CC, presented malignancy and poor prognosis [4]. The treatment methods of COAD mainly include surgery, radiotherapy and chemotherapy, and targeted drug therapy. With technological innovation and the application of new targeted drugs, the prognosis of COAD patients has been greatly improved, but the overall curative effect for advanced COAD is not good, and many patients resistant to the therapies [5]. Hence it becomes essential to explore underlying molecular mechanisms and identify novel targets for early diagnosis and precise treatment.

In this study, we first screened the DEGs from The Cancer Genome Atlas (TCGA) database. And we identified 2 DEGs through Stage I *vs.* Stage IV and Normal *vs.* Tumor. Finally, DNASE1L3 was selected through repeated verification of various databases. Further, we obtained the different expression of DNASE1L3 between cancer and adjacent cancer genes in each tumor sample from the TCGA database, and in many types of cancers, the expression was lower than normal tissue; DNASE1L3 was low expressed in COAD samples and associated with the stage. In predicting the correlation between COAD and DNASE1L3, the predictive ability of the variable DNASE1L3 has higher accuracy. Through GEPIA (http://gepia.cancer-pku.cn/), the low expression of DNASE1L3 was associated with poor overall survival of patients. Because immunotherapy is currently a hot topic in COAD research, we counted the number of neoantigens in each COAD and analyzed the relationship between DNASE1L3 expression and the number of neoantigens. Microsatellite Instability (MSI) represents the nucleotide insertions or deletions in the microsatellite loci. We analyzed the correlation between gene expression and MSI as follows, using Spearman's rank correlation coefficient. Then we conducted an immunological correlation analysis of DNASE1L3 in the TIMER website (https://icbi.i-med.ac.at/software/timiner/timiner.shtml), and we were surprised to discover that DNASE1L3 has an intimated connection with immune cells infiltration. We verified it using the TCGA database and got almost the same result. On the strength of this evidence, we speculated that DNASE1L3 is likely to be a prognostic biomarker and correlated with immune infiltration.

Finally, we verified the expression of DNASE1L3 in colon cancer by immunohistochemistry. The results showed that the expression of DNASE1L3 in colon cancer was lower than that in normal tissues, and it had a certain relationship with tumor stage.

Materials and Methods

TCGA database

The TCGA database contains exhaustive, multidimensional maps of key cancer genome changes in various cancers [6], which was selected for our study. All data have been collected and analyzed by the R language. Samples were then subjected to differential expression analysis using the edgeR package. Genes with log Fold-Change (FC)| >2 and P<0.05 were considered to be DEGs. We screened 12 DEGs from Stage I *vs.* Stage IV and 1614 DEGs from Normal *vs.* Tumor and integrated the DEGs through the VENE map. The expression of indicated genes in pan-cancer (contains 20 cancer types) and in different stages of COAD were collected from The Cancer Genome Atlas database (TCGA: https://www.cancer.gov/).

Kaplan–Meier analyses

For survival analyses, Kaplan–Meier plots and Hazard Ratios (HR) with 95% confidence intervals were determined using GEPIA 2 (http://gepia2.cancer-pku.cn/#survival) based on the expression status of indicated genes in TCGA data sets with median group cutoff.

Neoantigen prediction

Single nucleotide somatic variants were used to predict potential neoantigens using tumor-specific neoantigen detector (TSNAD) [7].

MSI prediction

PreMSIm is an R package for predicting microsatellite instability from the expression profiling of a gene panel in cancer. Therefore, we use the RNA-Seq cancer datasets for COAD cohorts from The Cancer Genome Atlas (TCGA) program to predict the relationship between the expression of DNASE1L3 and microsatellite instability [8].

TIMER

TIMER is a comprehensive resource for systematic analysis of tumor-infiltrating immune cells (https://icbi.i-med.ac.at/software/timiner/timiner.shtml). Therefore, we use the TIMER website to analyze the relationship between DNASE1L3 and immune cell infiltration.

Results

Identification of DEGs in COAD

After performing integrated analysis between tumor and normal tissues from the TCGA database, we screened 12 DEGs from Stage I *vs.* Stage IV and 1614 DEGs from Normal *vs.* Tumor. The DEGs (P<0.05 and |log(FC)| >2) from the dataset are shown in Figure 1A, 1B. We integrated the DEGs through the VENE map in Figure 1C. Red or green dots represent upregulated or downregulated genes, respectively.

The expression of DNASE1L3 in pan-cancer and COAD and its relationship with tumor stage

All tumor and adjacent normal tissues in TCGA were analyzed

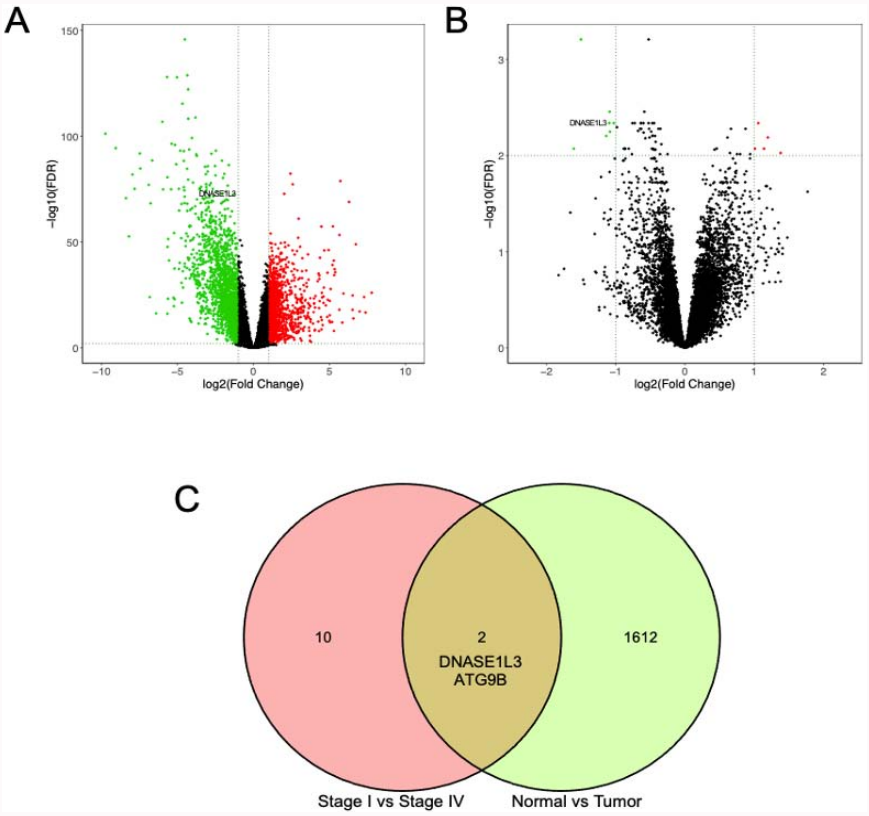

Figure 1: Identification of DEGs in the TCGA database. (A) Volcano plot of DEGs between COAD and normal control. (B) Volcano plot of DEGs between COAD Stage I and vs Stage IV control. (C) Interpretation of selected 12 genes and 1614 genes to identify possible candidate genes using Venn diagram; DNASE1L3 were selected.

to further comprehend the differential expression of DNASE1L3. The results revealed that DNASE1L3 expression was markedly decreased in BLCA, BRCA, CHOL, COAD, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA and USEC compared with adjacent normal tissues. However, DNASE1L3 expression was no statistical difference in CESC, ESCA, GBM and PAAD tissues compared with adjacent normal tissues (*p<0.05, **p<0.01, ***p<0.001) (Figure 2A). DNASE1L3 expression is lower in colon cancer than in normal tissues (Figure 2B). Besides, in predicting the correlation between COAD and DNASE1L3, the ROC showed that the expression of DNASE1L3 in COAD was 0.959 (AUC=0.959, CI = 0.923-0.995) (Figure 2C). The low expression of DNASE1L3 in COAD tended to be accompanied by an advanced tumor stage (Figure 2D).

Survival analysis of DNASE1L3 in COAD

To further research the survival value of DNASE1L3 in COAD, this study performed a survival assay according to TCGA COAD cohort data. As shown in Figure 3, low expression of DNASE1L3 was associated with worse prognosis of COAD patients and had a statistically significant effect (P<0.05) on patients' overall survival. Thus, DNASE1L3 may serve as a potential and novel biomarker for COAD.

The expression of DNASE1L3 is negatively correlated with neoantigens count

Cancer neoantigens derived from coding variants can be valuable candidates for developing cancer vaccines. T-cells can recognize neoantigens presented by cancer cells and eliminate them; which makes them valuable candidates for cancer immunotherapy. When the expression of DNASE1L3 is low, due to the weaker DNA hydrolysis ability, the expression of tumor mutation genes increases, and the number of tumors neoantigens increases. With the high expression of DNASE1L3, the ability of DNA hydrolysis is enhanced, the expression of mutant genes is less, and the number of tumors neoantigens is less (Figure 4).

The expression of DNASE1L3 is negatively correlated with MSI in COAD

Microsatellites (MSI) are DNA sequences composed of a single nucleotide or polynucleotide repeating sequences. The repeating unit is generally composed of 1 to 6 nucleotides. The copy number of the repeating unit determines the length of the microsatellite [9]. Microsatellite sequences are highly conservative and genetically stable. These sequences can affect the expression of certain cellular genes and directly or indirectly regulate the genome. They are located and connected to multiple important gene loci. These sites are not only related to human diseases but also directly related to etiology [10]. In the presence of MSI, the incidence of oncogene abnormalities increases, leading to the accumulation of a large number of gene mutations, and then the development of tumors [11]. Pan-cancer analysis of the relationship showed that DNASE1L3 is negatively correlated with MSI in COAD (Figure 5).

Tumor-infiltrating immune cells: The expression of DNASE1L3 is positively correlated with immune cells in COAD

DNASE1L3 is identified as a connection with immune cells

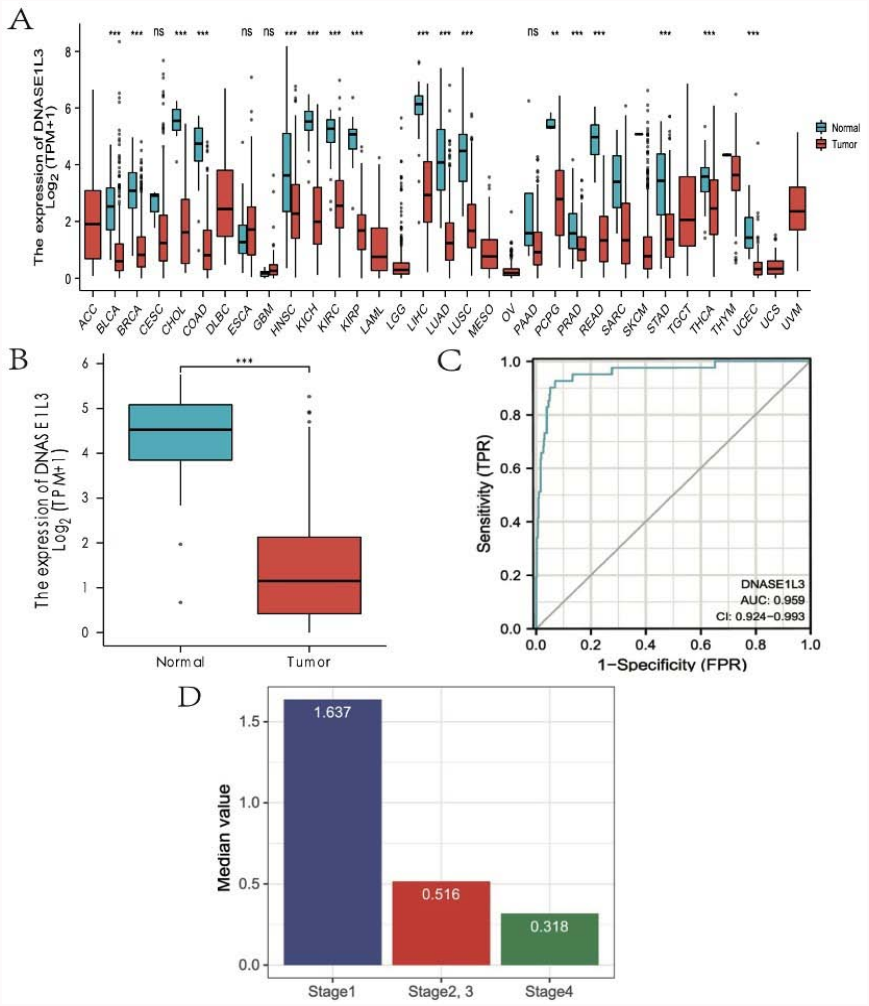

Figure 2: (A) Comparison of DNASE1L3 expression between pan-cancer cancerous and normal tissues based on TCGA. Red indicates tumor tissues and blue indicates normal tissues. *P<0.05, **P<0.01 and ***P<0.001. TCGA: The Cancer Genome Atlas; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast Invasive Carcinoma; CHOL: Cholangiocarcinoma; COAD: Colon Adenocarcinoma; CBM: Glioblastoma; HNSC: Head and Neck Cancer; KICH: Kidney Chromophobe; KIRC: Kidney Renal Clear Cell Carcinoma; KIRP: Kidney Renal Papillary Cell Carcinoma; LGG: Low Grade Glioma; LIHC: Liver Hepatocellular Carcinoma; LUAD: Lung Adenocarcinoma; LUSC: Lung Squamous Cell Carcinoma; PAAD: Pancreatic Adenocarcinoma; PRAD: Prostate Adenocarcinoma; READ: Rectum Adenocarcinoma; STAD: Stomach Adenocarcinoma; THCA: Thyroid Carcinoma; UCEC: Uterine Corpus Endometrial Carcinoma; ESCA: Esophageal Carcinoma.
(B) The expression of DNASE1L3 in COAD.
(C) The correlation between COAD and DNASE1L3 based on ROC.
(D) Associations between DNASE1L3 expression and stage in COAD based on TCGA.

infiltration. We analyzed the connection between DNASE1L3 and immune cells infiltration using two databases, and the results showed the high expression of DNASE1L3 is linked to higher infiltration rate in immune cells, containing B cells, CD8+ T cells, CD4+ T cells, macrophage, neutrophil, and dendritic cells (Figure 6).

The expression of DNASE1L3 was high in normal colon tissues, while low expression of DNASE1L3 was observed in COAD tissues

To determine the mRNA and protein expression of DNASE1L3 in colon adenocarcinoma, the DNASE1L3 expression from Tissue Microarray was analyzed by immunohistochemistry. As shown in Figure 7A, 7E, the expression of DNASE1L3 in normal colon tissue is higher than that in colon cancer tissue, and with the increase of tumor stage, the expression level shows a downward trend. As shown in Figure 7F, paired data analysis showed that the expression levels of DNASE1L3 in colon adenocarcinoma tissues (n=28) were significantly higher than those in adjacent tumor tissues (n=28) (P<0.001). According to the TNM staging classification, the rank sum test was performed between the data of normal colon tissue and each stage of COAD tissue. We found that the positive percentage of nuclei in normal tissues was the highest, and the data showed a downward trend with the increase of tumor stage. There are statistical differences between normal tissue and I (P=0.0312), II (P=0.0078), III (P=0.0156), and IV (P=0.0156) (Figure 7G).

Discussion

Colon Adenocarcinoma (COAD) is one of the leading causes of cancer-related deaths in the world, and in China, its incidence is increasing year by year. Due to the high frequency of recurrence and metastasis, the 5-year survival rate of COAD patients is only 30% [12]. Therefore, an in-deep investigation should be conducted to determine the underlying mechanisms of COAD and explore more novel potential targets for effective therapies against COAD.

Immunotherapy is the hottest cancer treatment method today,

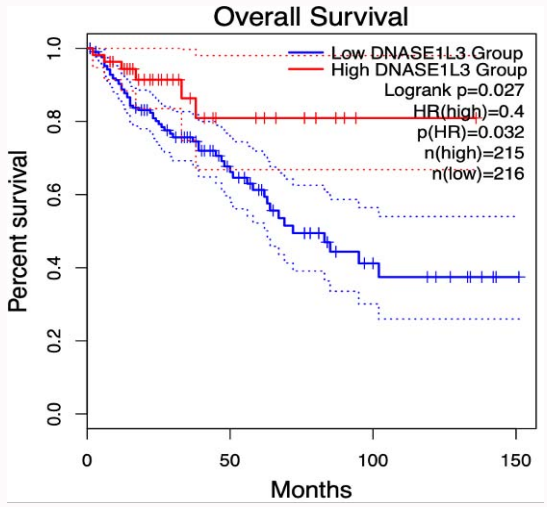

Figure 3: Diagnostic value of DNASE1L3 in COAD. K-M survival analysis was performed to determine differences in COAD between the low and high expression of DNASE1L3. Blue, low expression of DNASE1L3. HR: Hazard Ratio; p: Log Rank p-value.

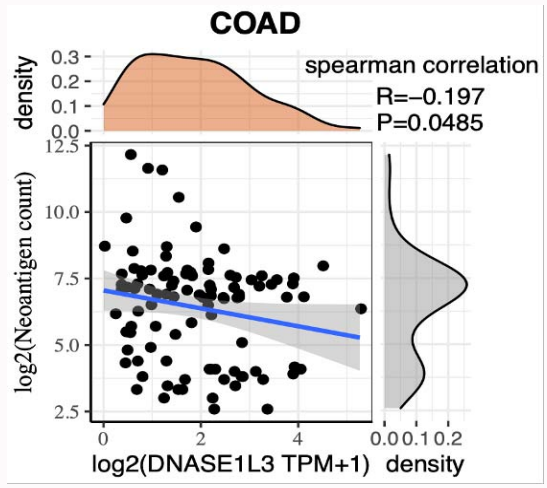

Figure 4: The relationship between the expression of DNASE1L3 and neoantigens or MSI. The expression of DNASE1L3 with neoantigen count.

and it is hailed as the third revolution in cancer treatment [13]. At present, immunotherapy is mainly divided into two methods. The first method is to use the effector cells/molecules of the immune system to directly attack tumor cells. This is called "passive" immunotherapy. The second method is to enhance the activation of the amplified immune system by regulating the endogenous immune regulation mechanism/immune activation mechanism, which is also called "active" immunotherapy [14]. The current status of immunotherapy in the COAD field can be simply summarized as "progress without breakthroughs". Only a few people are effective, and most patients still cannot benefit from immunotherapy [15]. Therefore, there is an urgent need to find targets for immunotherapy.

In this study, through database mining, we determined that DNASE1L3 is the DEG of COAD tumor and adjacent tissues and different tumor periods. Further, analyze the expression of DNASE1L3 in pan-cancer and in different COAD stages. Then studied the relationship with prognosis. And conducted exploratory research on gene expression and immune function.

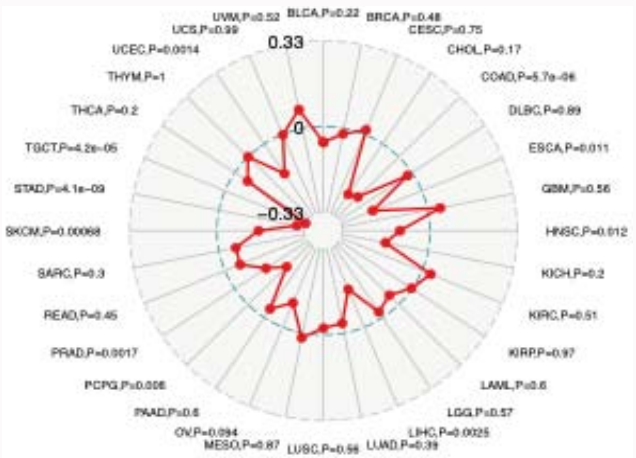

Figure 5: The expression of DNASE1L3 with MSI.

DNase1-Like 3 (DNASE1L3), also referred to as DNase γ, is a member of a family of four extracellular nucleases homologous to DNASE1 [16]. DNASE1L3 is a secreted DNASE1-like nuclease capable of digesting DNA in chromatin, and its absence causes anti-DNA responses and autoimmunity in humans and mice [17-20]. Previous studies have reported that the serum concentration of DNASE1L3 protein is reduced in dermatitis, SLE, and rheumatoid arthritis, which suggests that DNASE1L3 may be involved in the development of autoimmune diseases [17]. The current research involving DNASE1L3 mainly focuses on SLE. A group reported that DNASE1L3-deficient mice rapidly develop autoantibodies to DNA and chromatin, followed by an SLE-like disease [21]. However, there are still few other research reports on DNASE1L3. Recently, a study had found patients with positive DNASE1L3 expression had significantly longer overall survival, compared with patients with negative expression in HCC [22]. And there is no similar study in COAD. That low expression of DNASE1L3 with short OS and poor prognosis. DNASE1L3 is related to MSI, tumor neoantigens, and immune cell infiltration. Therefore, it has the potential to become a marker for judging the prognosis and the effectiveness of immunotherapy, and it may become a new target for immunotherapy in the future.

In recent years, there are increasing interest in exploring neoantigens for cancer immunotherapy [23]. Neoantigens are derived from non-synonymous mutations in the genome of tumor cells, so they have strict tumor specificity [24,25]. However, one of the challenges facing neoantigens-based immunotherapy is that new epitopes are usually not shared among cancer patients. And neoantigen load may form a biomarker in cancer immunotherapy and provide an incentive for the development of novel therapeutic approaches that selectively enhance T cell reactivity against this class of antigens [23]. We found DNASE1L3 has a negative relationship with the Neoantigens counts. We suspect that when DNAS1EL3 is under-expressed, the ability of DNA hydrolysis is weaker, the expression of tumor mutant genes increases, and the number of tumors neoantigens increases. The relationship needs further verification in order to guide tumor immunotherapy.

Microsatellite Instability (MSI) refers to the change in the number of repetitions of microsatellites caused by replication errors [26]. The mechanism is mismatch repair defects and 15% to 20% of colorectal cancer patients with high MSI [27]. According to the latest

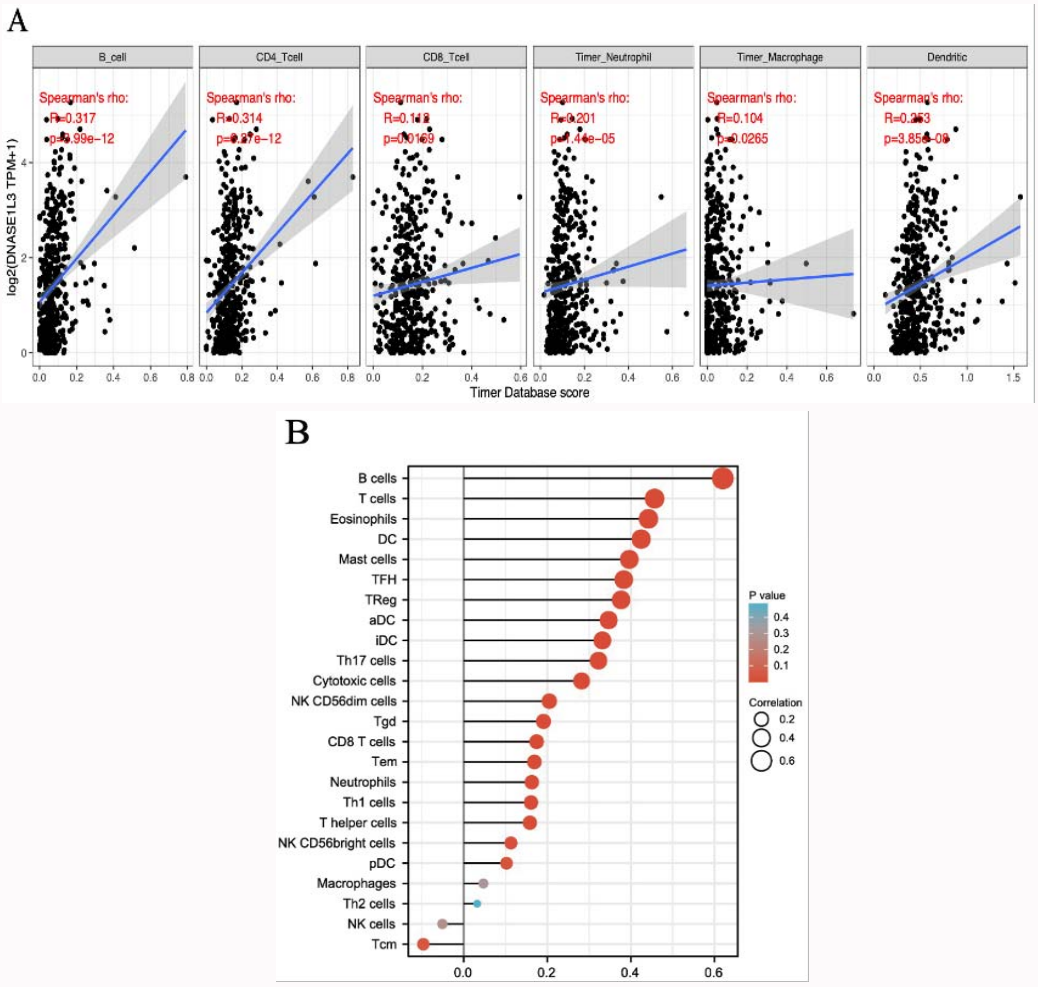

Figure 6: Correlation of DNASE1L3 expression with immune infiltration level in COAD (colon adenocarcinoma). (A) The correlation between the expression of DNASE1L3 in COAD and the level of immune cell infiltration in the TIMER database. (B) The correlation between the expression of DNASE1L3 in COAD and the level of immune cell infiltration in the TCGA database.

relevant guidelines issued by the National Comprehensive Cancer Network, it is recommended that all patients with colorectal cancer use polymerase chain reaction or immunohistochemistry for MSI/mismatch repair testing [28]. Colorectal cancer immune checkpoint inhibitor therapy is in the "MSI era" because MSI or Mismatch Repair gene status (MMR) is currently the best predictor of efficacy. Based on the MSI status, colorectal cancer patients can be divided into two groups according to the efficacy of immunotherapy: "dominant population"-MSI-H/dMMR colorectal cancer (MSI-H); "ineffective population" ——MSS/pMMR bowel cancer (MSS). Therefore, for MSI-H bowel cancer, immunotherapy will become an important part of the treatment. We found that DNASE1L3 is negatively correlated with MSI in COAD. The low expression of DNASE1L3 with a high degree of tumor malignancy, and the increase of MSI, the treatment is more effective. Therefore, we speculate that immunotherapy may be more effective for high malignancy COAD, and maybe less effective for low malignancy COAD. And the DNASE1L3 could be a co-testing method with MSI/mismatch repair testing to evaluate the efficacy of immunotherapy.

Tumor-Infiltrating Immune Cells (TIIC) are an indication of the host immune reaction to tumor antigens and act as a marker for prognosis in colorectal cancer [29]. In several solid tumors, the number of TIIC influences the prognostic and predictive power. In breast cancer patients, the ratio of TIIC in stroma lesions was correlated to patient prognosis [30]. Our result showed that DNAS1L3 had a connection with immune cells and is a potential target of immunotherapy.

In conclusion, the present study revealed that DNASE1L3 might be a prognostic biomarker and potential target of immunotherapy.

Conclusion

We processed a series of bioinformatics analyses to seek the DEGs. After repeated multiple validations, DNASE1L3 was identified as the potential prognostic biomarker. The low expression of DNASE1L3 is related to the low survival rate. This gene is also involved in the immune regulation system and positively correlated with immune cells in COAD. This gives us a new idea for further investigation.

Acknowledgement

Thanks to the grants mentioned earlier supporting the expense of the whole study. We also thank the colleagues of Liu's lab for providing valuable advice on experimental design and performance.

Funding

This study was supported by grants from the National Natural

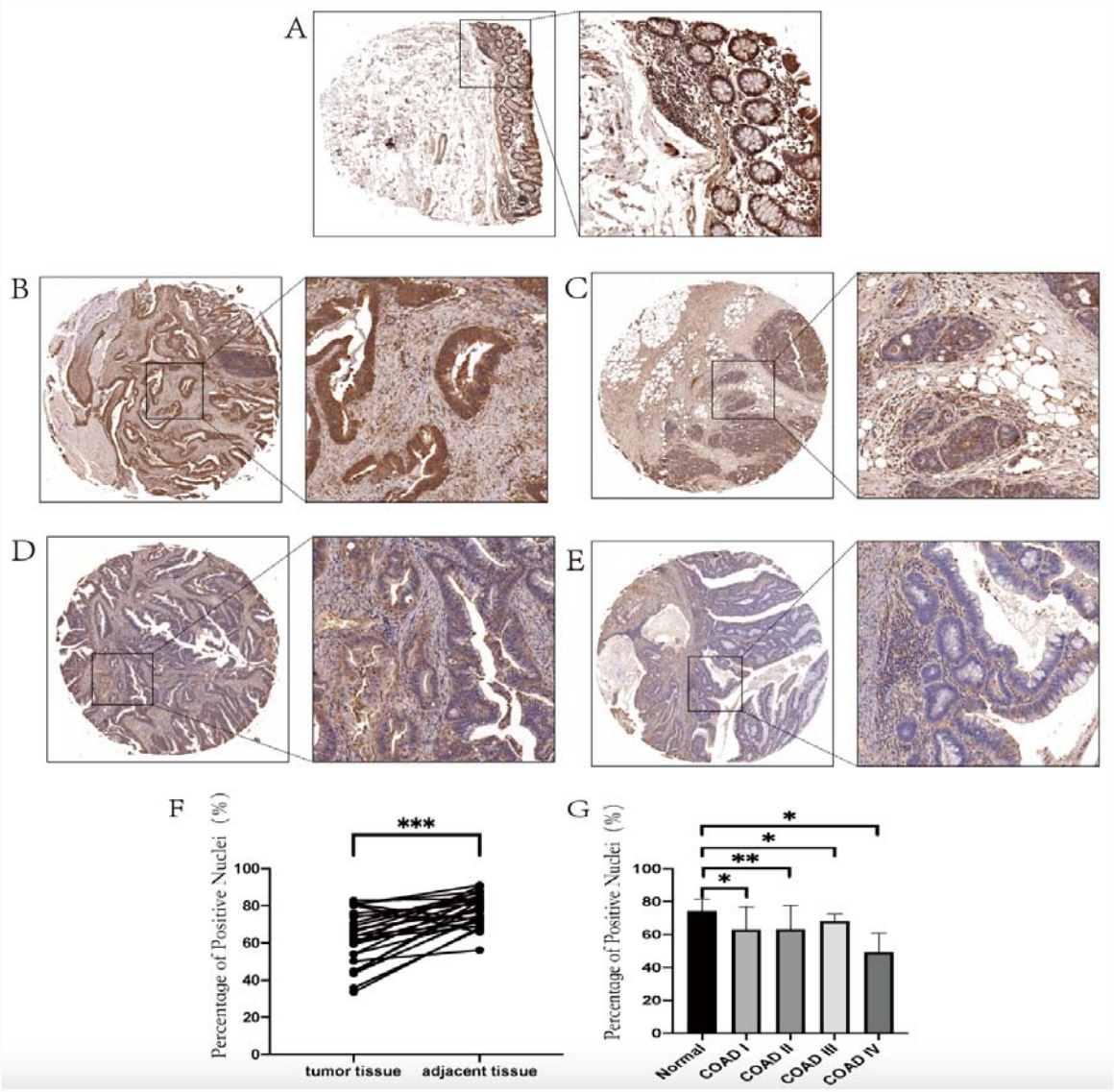

Figure 7: Immunohistochemistry. A-E are representative images of immunohistochemistry, A is normal colon tissue, B is COAD stage I tissue, C is COAD stage II tissue, D is COAD stage III tissue, and E is COAD stage IV tissue. F shows the positive percentage of nuclei obtained from the histochemical results of tumor tissue and adjacent tissue. The two groups were subjected to paired t-test (***, P<0.001). F shows the positive percentage of nuclei obtained from the histochemical results of normal colon tissue and each stage of COAD tissue. The normal group and COAD tumor stage groups were subjected to rank sum test (*, P<0.05; **, P<0.01).

Science Foundation of China (81973664), the Natural Science Foundation of Shenzhen Science and Technology Innovation Commission (JCYJ20190808140601638) and the Sanming Project of Medicine in Shenzhen (SZSM202111002).

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