Comprehensive analysis of the immune and prognostic implication of MASP1 in stomach adenocarcinoma


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Abstract. – OBJECTIVE: Stomach adenocarcinoma (STAD) is the major cancer worldwide with high morbidity and mortality rate. Late diagnosis and limited treatment options of STAD lead to disease progression, spread, and metastasis. Therefore, finding a new biomarker to diagnosis and treatment is very important for STAD in clinical practice.

MATERIALS AND METHODS: The clinical data, transcriptome data and CCLE data were downloaded from TCGA database and CCLE database, respectively. TIMER website, TISIDB website and CIBERSORT methodology were used to analyse immune infiltration. R software and R package were used to analyse gene difference expression, determine co-expression genes, conduct gene enrichment analyses, construct a prognostic signature and establish nomogram.

RESULTS: MASP1 was decreased in STAD compared with normal tissue at the mRNA level (p < 0.001). The enrichment analysis showed that mismatch repair (MMR) was related to the MASP1 gene. Up-regulation of MASP1 expression was positively associated with dendritic cells (p < 0.01), neutrophils (p < 0.05), macrophages (p < 0.001), CD4+ T cells (p < 0.001) and B cells (p < 0.05). A four-gene prognostic signature was determined based on MASP1-related immunomodulators. The prognostic signature was an independent prognostic predictor in STAD. Finally, we established a nomogram to forecast survival and the nomogram has a good prediction accuracy.

CONCLUSIONS: In STAD, MASP1 is closely related to immunity. MASP1 has the potential to positively regulate the abundance of immune cells. The MASP1-related prognosis signature and nomogram can accurately predict the survival of patients with STAD. Therefore, MASP1 is likely to be a diagnosis and promising immunotherapy target spot in STAD clinical practice.

Key Words: Stomach adenocarcinoma, STAD, Biomarker, MASP1, Immune, Prognosis.

Introduction

Stomach cancer is the major cancer worldwide, with approximately 769,000 deaths and beyond 1 million new cases by 2020, ranking fifth and fourth in global morbidity and mortality. The majority (approximately ninety percent) of stomach cancers are stomach adenocarcinomas (STAD), originating in the outer layer glands, or the mucosa, of the stomach. There are many main therapeutic strategies for gastric carcinoma, including surgery, chemotherapy, radiotherapy, immunotherapy and targeted therapy. However, current statistics show that median survival of patients with advanced gastric cancer disease is less than 12 months. Fortunately, the occurrence of immune-checkpoint inhibitors, such as programmed cell death 1 (PD-1), programmed death-ligand 1 (PD-L1), and antibodies against CTLA-4 were breakthroughs in the treatment of solid tumors. The achiever of immunotherapy has highlighted the importance of the tumor microenvironment (TME). TME richness of immune cells has been testified to correlate with survival and immunotherapy outcome in patients with solid tumors. Therefore, studying the interrelationship between biomarkers and immune cells may be a promising approach for STAD therapy. Mannose-binding lectin-associated serine protease 1 (MASP1) is a protein-coding gene located on chromosome 3q27.3. In humans, MASP-1 is
coded by a structural gene-MASP1. The encoded protein is synthesized as a proenzyme, which is activated when it binds to the ficolin, the mannos-binding lectin and the pathogen recognition molecules of the lectin pathway. This MASP-1 protein does not directly intervene in complement activation but acts as a complement activation enhancer by activating another complement serine protease or cleaving complement C2. Among its related pathways are the complement pathway and immune response lectin-induced complement pathway. Carroll et al. supported that complement deficiency impaired B and T cell responses. Kleczko et al. believed that the complement pathway could be used as a treatment strategy for lung cancer. These results indicated that MASP1 may be involved in the tumor immune microenvironment (TIME). However, the role of MASP1 in STAD remains unknown.

In this study, we focused on the immunological importance of MASP1 in STAD. The data were gained for detailed analysis from The Cancer Genome Atlas (TCGA) database. We explicitly examined the relationship between MASP1 and tumor immune-infiltrating, as well as MASP1-related immune pathways. Then, we constructed an immune prognostic feature using MASP1-related immunomodulators and verified its accuracy. Finally, we established a nomogram using immune prognostic features and clinicopathological features.

**Materials and Methods**

**Transcriptome and Clinical Datum**

We obtained datum on STAD from the TCGA database (https://portal.gdc.cancer.gov/). Datum included clinical and mRNA sequencing transcriptome datum. These data were processed with R software. The mRNA sequencing data contained 32 normal tissues and 375 tumor tissues. Gene expression data normalized to transcripts per million (TPM) were converted to log2 (TPM + 1) form. After processing clinical data through R software, we obtained 443 clinical cases. Then, we excluded the missing or insufficient data, cases such as gender, age, TNM stage, survival status and time, local infiltration and metastasis.

**Differential Expression and Co-expression of MASP1**

Tumor Immune Estimation Resource (TIMER) (https://cistrome.shinyapps.io/timer/) is an extensive gateway for systematically estimating immune infiltration in various cancers. The “DiffExp” module entails users detecting the differential expression of any interesting gene between all TCGA tumors and corresponding paracancerous tissue. We used the “DiffExp” module to detect the expression discrepancy of MASP1 across cancers. Additionally, we utilized R software to study the transcriptional data for acquiring the expression discrepancy of MASP1 in TCGA-STAD cohort. The Broad Institute Cancer Cell Line Encyclopedia is a database designed to supply detailed genomic information, computational analysis, and concretization for approximately 1,100 cell lines across 37 cancer types. The most common types of cancer were included in this program, such as stomach, breast, kidney, liver and lung cancer. To analyze MASP1 gene expression differences among cell lines, we downloaded the 37 cell lines expression matrix for STAD from CCLE dataset (https://portals.broadinstitute.org/ccle/about). We resorted to the R v4.0.5 software package ggplot2 to implement the above analysis. Additionally, the co-expressed genes of MASP1 were identified by R software. The filtering condition of the correlation test was corr > 0.5, and the filtering condition of the correlation test p-value was p < 0.05.

**KEGG and GO Analyses**

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Ontology (GO) annotation were conducted by the R clusterProfiler package with MASP1 co-expressed genes. Gene Ontology is a general resource for analyzing and interpreting high-throughput biological datasets. GO annotation notes knowledge of biological functions by affiliating GO terms to gene products. Both the gene product records and GO terms have machine-readable access numbers; thus, we can analyze large datasets by using these annotations. KEGG, comprising one computer-generated database and 15 manually managed databases, is a comprehensive database resource. KEGG was divided into four categories: Chemical Information, systems information, Health Information and Genomic Information.

**Detection of Immune Cells in TCGA STAD**

The Cell type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT) was used to identify and calculate 22 immune cells, consisting of NK cells, naive and memory B cells, myeloid subsets, plasma cells and T-cell
Comprehensive analysis of the immune and prognostic implication of MASP1

This approach mainly relies on a matrix of genetic markers of leukocytes, namely LM22. This document consists of 547 genes that tell the 22 human hematopoietic cells phenotype. We applied the CIBERSORT script obtained from the CIBERSORT website (https://cibersort.stanford.edu/) to perform the mRNA expression matrix analysis using CIBERSORT L22 as a reference. We utilize Monte Carlo sampling to compute the empirical deconvolution p-value for each case. After eliminating nonsignificant samples, the remaining samples were encompassed: 375 STAD vs. 32 normal samples.

Interrelationship between MASP1 and Tumor-infiltrating Immunologic Cells

The TIMER “Gene” module entails users studying the relevance between the expression of MASP1 and the abundances of 6 immunologic cells (neutrophils, dendritic cells, CD8+ T cells, CD4+ T cells, macrophages and B cells). Additionally, we utilized the “SCNA” module to learn about the interrelationship between the immune-infiltrating extent and somatic copy number alteration.

TISIDB (http://cis.hku.hk/TISIDB/) is a web resource for immune system and tumor interactions that combines different types of heterogeneous data. This web resource relied on collected and consolidated data from the following databases: TCGA, PubMed and other public databases. The TISIDB “Lymphocyte” module was used to obtain the interrelationship among the tumor-infiltrating lymphocytes (TILs) abundances and the MASP1 expression.

Immunomodulators

MASP1-related immunomodulators were obtained from the comprehensive knowledge base portal TISIDB. Immunostimulators and immunoinhibitors that were significantly involved in MASP1 gene expression were selected (Spearman correlation test, p-value < 0.05). Subsequently, the immunomodulators were used to create a protein-protein interaction (PPI) net with STRING (https://cn.string-db.org/). In addition, KEGG and GO analyses of these immunomodulators were accomplished by the WEB-based GEne SeT AnaLysis Toolkit (http://www.webgestalt.org/).

Prognostic Signature and Related Analysis

We established a prognostic signature using immunomodulators associated with MASP1. In Cox models, we adopted the Akaike information criterion to conduct the forward stepwise variable selection. After the related immunomodulators were settled, a risk score, in other words, the prognostic index, was formed: risk score = \( \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_i x_i \). Regarding the equation, \( \beta_i \) and \( x_i \) represent the risk coefficient and expression extent of the related immunomodulators originating from the Cox model, respectively. The relevance of clinical characteristics and prognostic signature with overall survival were appraised by log-rank test, univariate Cox analyses and K-M survival curve. Multivariate analysis was conducted for the prognostic index and adjusted for age, sex, stage and grade. The predictive precision of the prognostic index was appraised by the time-dependent receiver operating characteristic (ROC) curves with the survival ROC package.

Construction and Evaluation of Nomogram

For cancer prognosis, Nomograms are becoming increasingly common. To cater to the profile of an individual patient, Nomograms can transform statistical forecasting models into numerical assessments of individual event probabilities, for example, recurrence or death. In this study, we utilized clinical characteristics and prognostic index to design a nomogram based on the rms R package. Relying on the bootstrap method (1,000 replicates), we tried to use the concordance index (C-index) to weigh up the forecasting precision of nomogram. Plot calibration curves to convey the discrepancy of the forecasted probability and the actual probability of occurrence.

Statistical Analysis

R software version 4.0.5 performed the statistical analysis. Clinical factors interrelated with overall survival (OS) were appraised by multivariate and univariate Cox regression analyses. Only \( p < 0.05 \) indicated statistical significance, unless otherwise stated.

Results

Differential Expression of MASP1

To determine the difference in the expression of MASP1 in tumors and normal tissues, the “Diffexp” module of TIMER was used to analyze the levels of MASP1 mRNA in tumors and normal tissues. The result in Figure 1A shown that the
MASP1 expression in STAD (stomach adenocarcinoma), UCEC (endometrial cancer), THCA (thyroid cancer), READ (rectal adenocarcinoma), PRAD (prostate Adenocarcinoma), PCPG (pheochromocytoma and paraganglioma), LUSC (lung squamous cell carcinoma), LUAD (lung adenocarcinoma), LIHC (liver hepatocellular carcinoma), ESCA (esophageal carcinoma), COAD (colon adenocarcinoma), CHOL (cholangiocarcinoma), CESC (cervical squamous cell carcinoma and endocervical adenocarcinoma), BRCA (breast invasive carcinoma), BLCA (bladder urothelial carcinoma) were remarkably lesser than that in normal tissues. In order to further evaluate the expression of MASP1 in STAD, the TCGA RNA sequencing data were analyzed by using R software and revealed that MASP1 was significantly elevated in STAD tumor tissue (Figure 1B). Additionally, the results of the CCLE database showed that 97.3% (36 of 37) of STAD cell lines had low expression, and only the FU97 cell line expressed the opposite (Figure 1C).

Analysis Of Co-expressed Genes of MASP1

To further understand the potential role of MASP1 in STAD, we utilized CCLE datum to screen co-expressed genes by R software. The results of the co-expressed genes in Table I showed that MASP1 was positively correlated with 30 genes and negatively correlated with 15 genes. Additionally, the co-expressed genes were explored by enrichment analysis to further determine the potential role of MASP1 in STAD. GO annotation showed that 30 GO terms (10 MF terms, 10 BP terms and 10 CC terms) were enriched (Figure 2A). KEGG pathway analysis (Figure 2B) showed that MASP1 participated in 9 pathways, including “mismatch repair” and “gastric cancer”.

Immune Cells Landscape in Normal and STAD Tissues

To explore the immune infiltration in STAD, the CIBERSORT methodology and R software were conducted to obtain the distribution of distinct immune cells between malignant tissues and corresponding normal tissues in STAD. Contrasted with normal tissues, the infiltration abundance of macrophages M1, macrophages M0 and B cells naive increased in STAD tumor tissues, while, the infiltration abundance of neutrophils, B cells memory, T cells CD8, mast cells resting, eosinophils, monocytes, plasma cells and T cells CD4 memory resting declined in tumor tissues (Figure 3A). Figure 3B shows different modes of immune cell distribution. Moreover, the interrelation in the immune cells was revealed in STAD (Figure 3C).

Relationship Between MASP1 and Immune Infiltration

Through the results of the previous step, we found that a difference existed in the immune cells between normal and malignant tissues in STAD. Therefore, we tried to determine whether MASP1 expression was involved in the immune cells. The correlations were roughly analyzed by TIMER “Gene” module. The result showed that increased MASP1 expression positively correlated with five types of immune cell infiltration abundance (Dendritic Cell, CD4+ T Cell, Neutrophil, B Cell, Macrophage) (Figure 4A). According to Figure 4B, we found that SCNA of MASP1 varied among the abundances of six immune infiltrates. Additionally, we found that the TILs were positively or negatively correlated with the MASP1 mRNA level (Figure 5).

To explore the potential immune modulatory function of MASP1 in STAD, TISIDB was used to study the association between immunomodulators and MASP1. We identified 30 immunostimulators (ULBP1, TNFSF9, TNFSF18, TNFSF15, TNFSF14, TNFSF13, TNFRSF8, TNFRSF25, TNFRSF18, TNFRSF17, TNFRSF14, TNFRSF13C, TNFRSF13B, TMEM173, RAET1E, PVR, MICB, IL6R, IL6, ICOS, ENTPD1, CXCR4, CXCL12, CD70, CD48, CD40LG, CD40, CD28, CD27, C10orf54) and 14 immunoinhibitors (VTNC1, TGFB1R1, CSF1R, PVRL2, IL10, KDR, TGFBR1, PEDCD1LG2, IL10RB, LGALS9, CD244, CD160, BTLA, ADORA2A) that were statistically correlated with MASP1 expression in STAD (Figure 6A). The PPI network was formed depending on these 44 immunomodulators in STRING (Figure 6B). Additionally, we used MASP1-related immunomodulators for functional enrichment analyses by the WebGestalt website. The KEGG pathway enrichment analysis of these immunomodulators suggested that Natural killer cell mediated-cytotoxicity, T-cell receptor signaling pathway and Th17-cell differentiation were correlated with MASP1-mediated immune events (Figure 6C). GO annotation was also applied to interpret these immunomodulators (Figure 6D).

Construction of Gene Prognostic Signature

To explore the prognosis value of MASP1-related immunomodulators in STAD, we conducted a stepwise Cox multiple regression analysis.
Figure 1. Expression and alteration of MASP1 in tumor or tissue. **A**, Expression of MASP1 from a pancancer view. **B**, Differential MASP1 expression in STAD and matching normal tissue by TCGA database. **C**, MASP1 expression in 37 STAD cell lines. Data were obtained via the CCLE dataset.
Relying on the TCGA-STAD cohorts, the best four prognostic characteristic genes were determined, and the correlation between OS and these genes was also analyzed and evaluated (Figure 7A). Then, the risk score was determined depending on the proposed equation. The risk coefficients of TGFB1, TNFSF18, IL6 and CXCR4 were 0.23, -0.37, 0.11 and 0.15, respectively. Subsequently, the median of the risk score divided patients into a high-risk group and a low-risk group.

According to Figure 7B, the K-M survival curve revealed that low risk patient was more likely to survive longer than high-risk patient \((p\text{-value} < 0.001)\). To estimate the accuracy of the prognosis signature, the ROC curve was used. The results revealed that the area under the curve (AUC) scores for stage, age, and risk score were 0.629, 0.592 and 0.659, respectively. Combining the risk score with stage and age, the AUC was 0.719 (Figure 7C). Moreover, Figure 7D shows the landscape of gene expression profiles, survival status and risk scores. Next, the risk score was further analyzed through the univariate and multivariate Cox regressions. The risk score was evidently associated with OS (HR=2.038, 95% CI=1.457-2.857, \(p\text{-value} < 0.001\)) in the univariate Cox regression models. At the same time, after adjustment for stage, gender, grade and age, the risk score was an independent prognosis predictor in STAD assessed by multivariate Cox regression (HR=2.457, 95% CI=1.677-3.600, \(p\text{-value} < 0.001\)) (Figure 7E).

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Figure 2. The co-expressed genes of the MASP1 gene were analyzed. There were 30 GO terms (A) and 9 KEGG pathways (B).
Figure 3. Identification and quantification of 22 immune cells by the CIBERSORT method. The distinctions of the immune cells distribution between normal (blue) and tumor (red) tissues in STAD were shown in violin plot (A) and heatmap (B). The interrelationships in the 22 subsets of immune cells were different in the STAD cohorts (C).
Figure 4. The relevance between immune cells and MASP1 in STAD based on TIMER. A, The interrelationship between 6 kinds of immune cells and MASP1 expression. B, Distinction of the extent of immune infiltration in STAD with MASP1 SCNA.
Figure 5. Evaluations among immune cell subset extent and MASP1 by TISIDB.
Figure 6. Identification and analysis of MASP1-related immunomodulators. A, Heatmaps of the interrelationships of MASP1 with immunoinhibitors (right) and immunostimulators (left) in STAD. B, PPI network of 44 MASP1-related immunomodulators. The KEGG pathway analysis C, and GO annotation D, of the network.
Figure 7. The building of the prognosis signature and the prognostic values of risk scores. A, The hazard ratios of 4 genes are displayed in the forest plot. B, K-M curves regarding the risk score. C, Time-dependent ROC curves at 3-years for STAD. D, The landscape of gene expression profiles, survival status and risk scores for STAD. E, Univariate and multivariate Cox regression analyses of the risk score in STAD.
Figure 8. Formation of the prognosis nomogram in TCGA database. A, Nomogram for forecasting the 1-, 3-and 5-year OS in STAD patients. B-D, OS calibration curves for STAD patients at 1, 3, and 5 years. The X and Y axes represent the nomogram-predicted OS and the actual OS, respectively.


**Construction of Nomogram**

Finally, we established a predictive-nomogram after coordinating T, N, M, age, stage and risk score (Figure 8A). The prognostic nomogram C-index was 0.608. By determining the scores of each patient, we forecasted the likelihood of OS at 1, 3, and 5 years. The calibration curves showed that the nomogram had an outstanding predictive effect on OS (Figure 8 B, C, D) in STAD patients.

**Discussion**

Recently, tumor therapy has changed greatly under the influence of immunotherapy. By directing the immune system to distinguish and devastate cancer cells, some patients can expect potential recovery and deep and long-term remission\(^{30}\). For some solid cancers, such as lung cancer and melanoma, immunotherapy can clearly improve the response to treatment\(^{30}\). The immunotherapy approach for gastric cancer has displayed some inspiring results and has reformed the treatment process. Novel immunotherapies targeting new molecules and various combination therapies have now been proposed. However, the disease is poorly prognosed\(^{31}\). Actually, limited treatment options and late diagnosis of gastric cancer led to disease progression, spread, and metastasis\(^{4}\). Therefore, finding new biomarkers and therapeutic targets for the immune system has become an urgent task for STAD in clinical practice.

In our study, the MASP1 expression was down-regulated, compared with that in normal tissue, in STAD. Next, we found that MASP1 was linked to the immune pathway and tumor immune infiltration. Furthermore, relying on MASP1-related immunomodulators, we performed stepwise Cox regression models to recognize 4 gene risk-predictive signature. Finally, we built a prognostic nomogram relying on the risk prediction signature and clinical features.

Through TIMER analysis and TCGA data analysis, we found that MASP1 expression was down-regulated in STAD tissue compared with normal tissue. This difference suggests that MASP1 may have some connection to STAD, but the function of MASP1 in STAD is uncertain. To explore the role of MASP1 in STAD, we found some co-expressed genes and performed functional enrichment analyses. The results of enrichment analysis showed that MASP1 was related to the “mismatch repair” and “gastric cancer” pathways. DNA mismatch repair (MMR) is a biological conservative system for the detection and repair of base additions, deletions, and defective insertions that occur during some forms of DNA damage, DNA recombination, and DNA replication\(^{32}\). Previous literature documented that MMR could prevent mutation accumulation by inducing apoptosis or controlling cell cycle checkpoints\(^{33}\). Willis et al\(^{34}\) supported that MMR abundance was bound up with the density of TILs. Recent studies have demonstrated that MMR is a successful and actionable biomarker for immune checkpoint inhibitor (ICI) therapy\(^{35}\). This evidence suggests that MASP1 probably plays an immunological role in STAD.

To understand the immune role of MASP1 in STAD, we first explored the compositions of intratumoral immune cells of each tumor sample. We found that the formation of 22 immune cells in TIME was remarkably varied in STAD when contrasted finely with normal tissues using CIBERSORT analysis. This difference was the premise that we can continue to conduct further immune-related analysis. A significant result of this study was the influence of MASP1 on the infiltration of STAD immune cells. The TIMER analysis showed that the MAPS1 expression was positively related to the abundances of Dendritic Cell, CD4+ T Cell, Macrophage, B Cell and Neutrophil. Copy number reduction resulted in decreased infiltrating-degree of dendritic cell, neutrophil, macrophage, CD8+ T cell, CD4+ T cells and B cell. It has been documented that CD8+ and CD4+ T cells were a positive prognostic factor by controlling tumor growth\(^{36}\). Dendritic cell can also improve prognosis by improving the effectiveness of cancer immunotherapy\(^{37}\). Although B Cell\(^{38}\), Macrophage\(^{39}\) and Neutrophil\(^{39}\) have antitumorigenic and protumorigenic forms, we believe that MASP1 has a positive effect on TIME. Therefore, we need to exert the anti-tumor effect of B cell, macrophage and neutrophil by regulating MASP1. In addition, the enrichment analysis of the MASP1-related immunomodulators suggested that Natural killer cell mediated-cytotoxicity, T-cell receptor signaling pathway and Th17-cell differentiation were correlated with MASP1-mediated immune events. Natural killer cell mediated cytotoxicity has been shown to have anti-tumor effects\(^{41}\). This evidence further confirmed that MASP1 played an active immunological role in STAD. For various cancers, gene prognostic signatures have been increasing widely used as prognostic factors. Zhang et al\(^{42}\) created a stable genetic signature associated with 6 immune gene and set up a valid prognostic nomogram, which was capable of conducting overall sur-
vival prognosis and risk stratification in low-grade gliomas. Wu et al. established a 3-gene signature and confirmed it by ROC curves in liver hepatocellular carcinoma. In this study, we used a stepwise Cox regression model to identify a 4-gene risk-predictive signature relying on MASP1-related immunomodulators. The signature was favorable and remarkably associated with survival in TCGA-STAD cohorts. The prognostic index was an independent prognostic predictor after being assessed by multivariate Cox regression. Then, a prognostic nomogram was established after coordinating T, N, M, age, stage and risk score, and the prognostic nomogram C-index was 0.608. The outcomes of this study may benefit physicians with a practical and accurate prognostic method for STAD patients.

While the current study has some advantages, limitations should be noted. First, we all used public data for analysis. We should further confirm the accuracy and practicability of the results through experiments on MASP1 expression and the correlation with immunity. These results need to be confirmed in internal patient populations. Second, the bond between immunotherapy response and MASP1 expression could not be analyzed due to insufficient data in databases.

Conclusions

Taken together, our outcomes implied that MASP1 may produce a marked effect in controlling the TIME of STAD. The prognostic signature stemming from MASP1-associated immunomodulators was an independently predictive of overall survival in STAD. The prognosis nomogram could be used to predict OS in STAD and aid in adjusting treatment and making medical decisions.

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Author Contributions

Chaohua Zhang had a substantial contribution to conception and design of the study and drafted the article. Zhiquan Xu performed the acquisition, analysis, and interpretation of data. Linglong Peng conducted a supervision in the study. Jijian Wang made some critical revisions related to relevant intellectual content of the manuscript. Haitao Gu performed the validation and final approval of the version of the article to be published.

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Conflict of Interest

The authors declare that they have no conflict of interest to declare.

Data Availability Declaration

The datasets analyzed during the current study are available in the repository TCGA website (https://portal.gdc.cancer.gov).

Ethics Statement

Since the data in the TCGA database were public, informed or ethical consent was not necessary for this study.

References


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