Original Article

Comprehensive analysis of the correlation between GSTM1 and tumor immunity in colon cancer

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Background: Glutathione S-transferase mu 1 (*GSTM1*) is one of the major glutathione conjugation enzymes. Its expression and activity have been suggested to correlate with the occurrence of colon cancer; however, the role of *GSTM1* in tumor immunity remains unclear.

Methods: Relevant data downloaded from The Cancer Genome Atlas (TCGA), Clinical Proteomic Tumor Analysis Consortium (CPTAC), and Human Protein Atlas (HPA) was used to perform a multi-dimensional expression analysis of *GSTM1* in colon adenocarcinoma (COAD). The correlation between *GSTM1* and tumor immunity was analyzed with multiple online tools. Then protein-protein interaction (PPI) network and functional enrichment analyses of *GSTM1*-associated immunomodulators were performed. Further, we developed the Cox regression model based on the *GSTM1*-related immunomodulators. Finally, a *GSTM1*-based clinical nomogram and a calibration curve was established to predict the probability and accuracy of long-term survival.

Result: *GSTM1* was significantly downregulated in COAD versus normal tissues. Infiltration levels of B cells, $CD8^+$ T cells, and dendritic cells were closely correlated to *GSTM1* gene copy number deletion, and *GSTM1* expression levels in COAD positively correlated with dendritic cell, B cell, neutrophil, and macrophage infiltration. Functional enrichment analysis indicated 36 *GSTM1*-related immunomodulators are involved in immune-related pathways of regulating T cell activation and lymphocytic activation. A 2-gene prognostic risk signature based on the 36 *GSTM1*-related immunomodulators was built using the Cox regression model, and the risk signature in combination with stage had an area under the curve (AUC) value of 0.747 by the receiver operating characteristic method. patients with higher risk scores—calculated based on 2 gene prognostic risk characteristics and further identified as an independent prognostic factor—were associated with worse survival using the Kaplan-Meier analysis. Together, the clinical nomogram and calibration curve based on *GSTM1* suggested a good prediction accuracy for long-term survival probability.

Conclusions: Our study provided evidence supporting the significant role of *GSTM1* in COAD immunity and suggests *GSTM1* as a potential novel target for COAD immunotherapy.

Keywords: Glutathione S-transferase mu 1 (GSTM1); colon adenocarcinoma (COAD); immune cells; prognosis; nomogram

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Introduction

Colon cancer is a common form of cancer with a multifactorial etiology affected by both genetic and environmental factors (1). While comprehensive surgical treatment is emphasized for colon cancer, treatment options for patients with advanced stages are very limited (2) and outcomes remain poor (3). The prognosis of colon cancer is poor, with low postoperative survival and high recurrence rates (4). New treatments to improve the prognosis of colon cancer have focused on immunotherapy strategies (5,6). Recent discoveries regarding the tumor microenvironment and the evasion of immune destruction (7) suggest immunotherapy as a precision treatment model, which may provide effective and alternative treatment approaches (8).

The immune checkpoint is a molecular hallmark to protect our immune system, which normally occurs through inhibition of T cell differentiation and proliferation to maintain the immune balance. Overexpression of the immune checkpoint molecules in tumor tissue inhibits the activation and proliferation of T cells and induces apoptosis of T cells, leading to the formation of an immunosuppressive tumor microenvironment and allowing tumor cells to escape immune monitoring (9). Immune checkpoint therapy has been shown to block inhibitory checkpoints and restore effective T cell function (10); Colon cancer was the first human tumor to be found to be immune-monitored by an adaptive immune response, and the immune system plays a complex role in colon cancer tumor immune evasion and tumor progression (11). Immune cell infiltration has been shown to have better prognostic value than classic tumor invasion criteria. While targeting the immune system with immune checkpoint inhibitors has surprising effects on some cancers, it has limited efficacy for colon cancer treatment due to the strong resistance of tumors against immune infiltration (12-14). A comprehensive understanding of colon cancer immunology and its molecular regulatory mechanism is necessary to ensure the success of immunotherapy.

Glutathione S-transferases (GSTs) are proteins that protect against oxidative stress caused by substances such as reactive oxygen species (ROS) (15). While one feature that distinguishes cancer cells from normal cells is that they can produce more ROS (16). ROS play a key role in cell signaling, cell damage, immune responses in promoting the occurrence and development of tumors (16-19). The glutathione S-transferase M1 (*GSTM1*) gene has been studied extensively in cancer due to its polymorphisms that are associated with tumor prognosis (20). *GSTM1* can inhibit the activity of apoptosis-regulatory kinase 1 (*ASK1*) (21), an MAP kinase that induces the death of cytotoxic tumor cells by activating the JNK and p38 pathways (22-24). Multiple studies have indicated that a lack of *GSTM1* may contribute to the occurrence of malignant tumors, including colon cancer (25,26), liver cancer (27), and breast cancer (28). Reported experimental results have suggested *GSTM1* could be a potential target for colon cancer treatment; however, direct evidence is needed to reveal the role of GSMT1 in colon cancer tumor immunity to support this notion.

We investigated the potential manipulatory role of *GSTM1* in colon adenocarcinoma (COAD) to propose potential *GSTM1*-based immunotherapy. Herein, we evaluated the expression of *GSTM1* and its relationship with immune cell infiltration in 521 cases of COAD and further screened *GSTM1*-related immunomodulators. We then established prognostic risk signatures based on identified immunomodulators and calculated the relevant risk scores. Lastly, we constructed a nomogram by integrating the immune signature and other clinical features as prognostic biomarkers to predict the probability of long-term survival of COAD patients. We present the following article in accordance with the TRIPOD reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1060/rc).

Highlight box

Key findings

- GSTM1 plays an important role in the tumor immunity of COAD;
- Risk model of GSTM1-associated immunomodulators has a certain guiding value for the prognosis of COAD patients.

What is known and what is new?

- GSTM1 plays an important role in tumorigenesis and development as glutamate conjunction enzymes;
- GSTM1 is closely related to tumor immune related cells in COAD. The prognostic risk model of GSTM1-related immunomodulators was considered to be an independent prognostic factor for patients with COAD, and had good predictive accuracy for long-term survival probability.

What is the implication, and what should change now?

- GSTM1 may play a protective role in COAD by affecting tumor immunity. Further *in vitro* and *in vivo* studies were needed to verify the relevant mechanisms.

Methods

Case resources and expression analysis

Transcriptional expression RNA-sequencing (RNA-seq) profile data and clinically related data were downloaded from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/). Excluding samples with incomplete data, we enrolled 14 cancer types, including at least 10 samples in the normal group. A total of 521 cases from TCGA-COAD were included, which contained 480 cancer tissues and 41 normal tissues. Furthermore, a log2 transformation was performed on the RNA-seq data in the Fragments Per Kilobase per Million (FPKM) format. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

UALCAN (http://ualcan.path.uab.edu/) is an effective online analysis and mining website for cancer data, which can be used to perform biomarker identification, expression profile analysis, and survival analysis of human genes. In this study, we conducted a comprehensive analysis of the *GSTM1* protein expression data (29,30).

Correlation between GSTM1 and immune cell infiltration

ROC plotter is an online tools that can link transcriptome level data of multiple tumors with gene expression and therapeutic response (https://www.rocplot.org/) (31). The "immunotherapy" module was used to investigate the correlation between the response of 1,434 patients receiving any form of immunotherapy and the expression of GSTM1. Then we investigated the correlation between the response of patients receiving treatment and the expression of GSMT1 in various immunotherapy subgroups, including anti-programmed death 1 (PD-1) therapy, anti-programmed cell death-ligand 1 (PD-L1) therapy, and anti-cytotoxic T lymphocyte-associated antigen-4 (CTLA4) therapy. The Tumor Immune Estimation Resource (TIMER) is an online database for systematically analyzing immune cell infiltration of different cancer types (cistrome.dfci.harvard.edu/TIMER/). It contains genes from cancer histograms (TCGA) of 32 cancers focused on the correlation and survival analysis between gene expression, mutant genes, and immune infiltration abundance (32). We used the "GENE" module of TIMER to evaluate *GSTM1* expression and infiltration of the six types of immune cells in COAD. Then, the "SCNA" module of TIMER was conducted to explore the correlation between somatic copy number changes and the abundance of immune infiltration in COAD.

Screening and comprehensive analysis of GSTM1-related immunomodulators

We extracted immunomodulators that were significantly correlated with *GSTM1* through TISIDB (http://cis.hku.hk/TISIDB/) (33). Furthermore, in this study, we used the STRING database (http://string-db.org/) to construct a protein-protein interaction (PPI) network by *GSTM1*-related immunomodulators (high confidence, 0.700). We aimed to understand the working principle of each protein, the reaction mechanism of biological signals and energy substance metabolism under pathological conditions, and the functional relationship between proteins (34). Additionally, functional enrichment analysis of the co-expression gene set was performed with the clusterProfiler package (V 3.14.3) and further visualized with the ggplot2 package (V3.3.3) (35).

Establishment and evaluation of a prognostic risk model

We further determined a prognostic multiple immune gene signature out of the *GSTM1*-associated immunomodulators. Single-factor Cox analysis was used to identify immune prognostic-related genes ($P<0.05$), and multivariate Cox regression analysis was used to finally develop an immune-related prognostic risk model (36). Patients were divided into high- and low-risk groups using the median risk score as the cutoff value. The Kaplan-Meier survival curve was performed for the risk model prognosis analysis. The prognostic accuracy of the risk score was determined by the time-dependent receiver operating characteristic (ROC) curve using "time ROC" packages. The risk curve of the model was used to assess the significance of the difference in survival between the high- and low-risk groups (37). Additionally, multivariate Cox analysis was performed after adjusting for age, sex, and stage to verify the independent prognostic implications of the risk score.

We assessed the individual prognosis of TCGA-COAD patients using nomogram, which is based on multivariate regression analysis and integrates multiple predictors to express the relationship between variables in the prediction model (38). In this study, based on the result of multifactor regression analysis, we used the "RMS" R package [6.2-0 version] to construct a nomogram to predict the possible overall survival (OS) of the individual patients at one year, three years, and five years, including multiple predictors such as gender, age, Tumor-Node-Metastasis (TNM) stage, and risk score. and the relationships between these variables

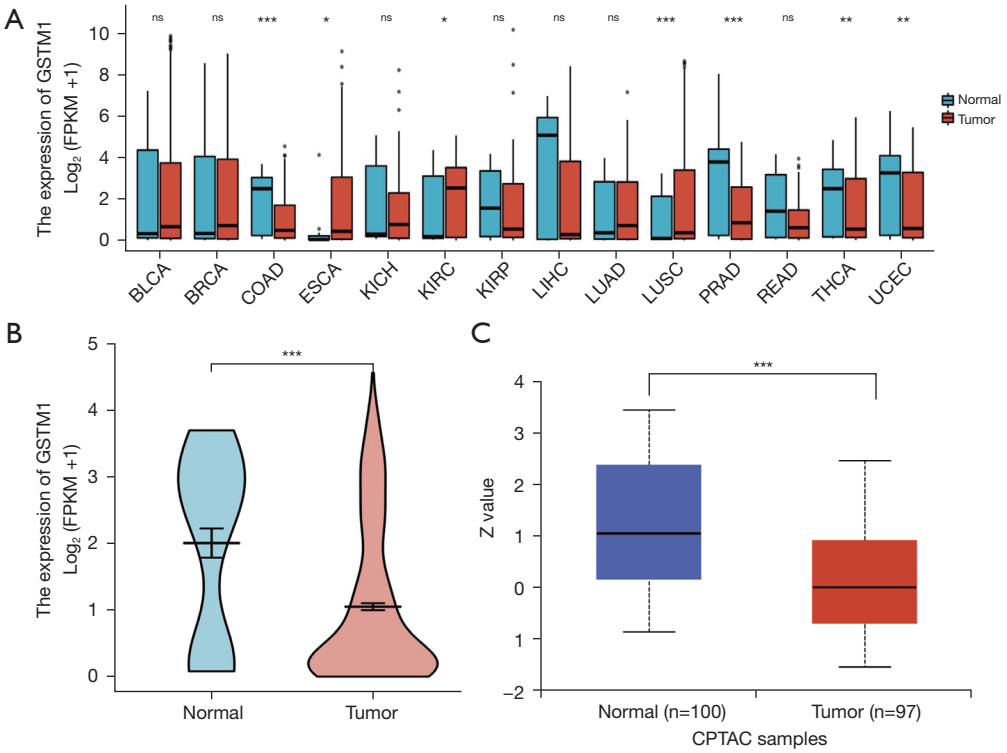


Figure 1 The mRNA and protein expression levels of *GSTM1*. (A) The expression model of *GSTM1* in pan-cancer. mRNA expression of *GSTM1* is downregulated in 4 of the 14 cancer types. (B) The mRNA expression level of *GSTM1* was assessed in 480 TCGA-COAD samples and 41 TCGA normal samples. (C) The protein expression Z-value of *GSTM1* in COAD analyzed by CPTAC. *, P<0.05; **, P<0.01; ***, P<0.001. BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; CPTAC, Clinical Proteomic Tumor Analysis Consortium; ESCA, esophageal carcinoma; FPKM, fragments per kilobase per million; GSTM1, glutathione S-transferase mu 1; KIRP, kidney renal papillary cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; ns, not significant; READ, rectum adenocarcinoma; TCGA, The Cancer Genome Atlas; THCA, thyroid carcinoma; PRAD, prostate adenocarcinoma; UCEC, uterine corpus endometrial carcinoma.

were transformed into a visual graph to make the results of the predictive model more readable.

Statistical analysis

Statistical analyses were conducted by R V 4.0.5 and V 3.6.3. Visualization was performed using ggplot2 (V 3.3.3). The Mann-Whitney U and Z tests were performed to identify the *GSTM1* expression level. Survival curves were generated using the Kaplan-Meier method. The correlational analysis of gene expression was performed by the Spearman method. Univariate and multivariate analyses were conducted using Cox regression models to determine independent prognostic factors. A P value <0.05 was considered statistically significant.

Results

Downregulation of GSTM1 mRNA and protein expression in COAD

We searched and analyzed a dataset of 14 cancers in the TCGA database with the filter condition that the normal tissues of each cancer contained at least 10 samples, and the mRNA expression level of *GSTM1* in different types of cancer was estimated. As shown in *Figure 1A*, compared with normal tissues, the expression of *GSTM1* was significantly downregulated in COAD, gastric adenocarcinoma, prostate cancer, thyroid cancer, and endometrial cancer. These results are consistent with previous meta-analysis results (25,27,39,40).

We further analyzed the mRNA and protein expression

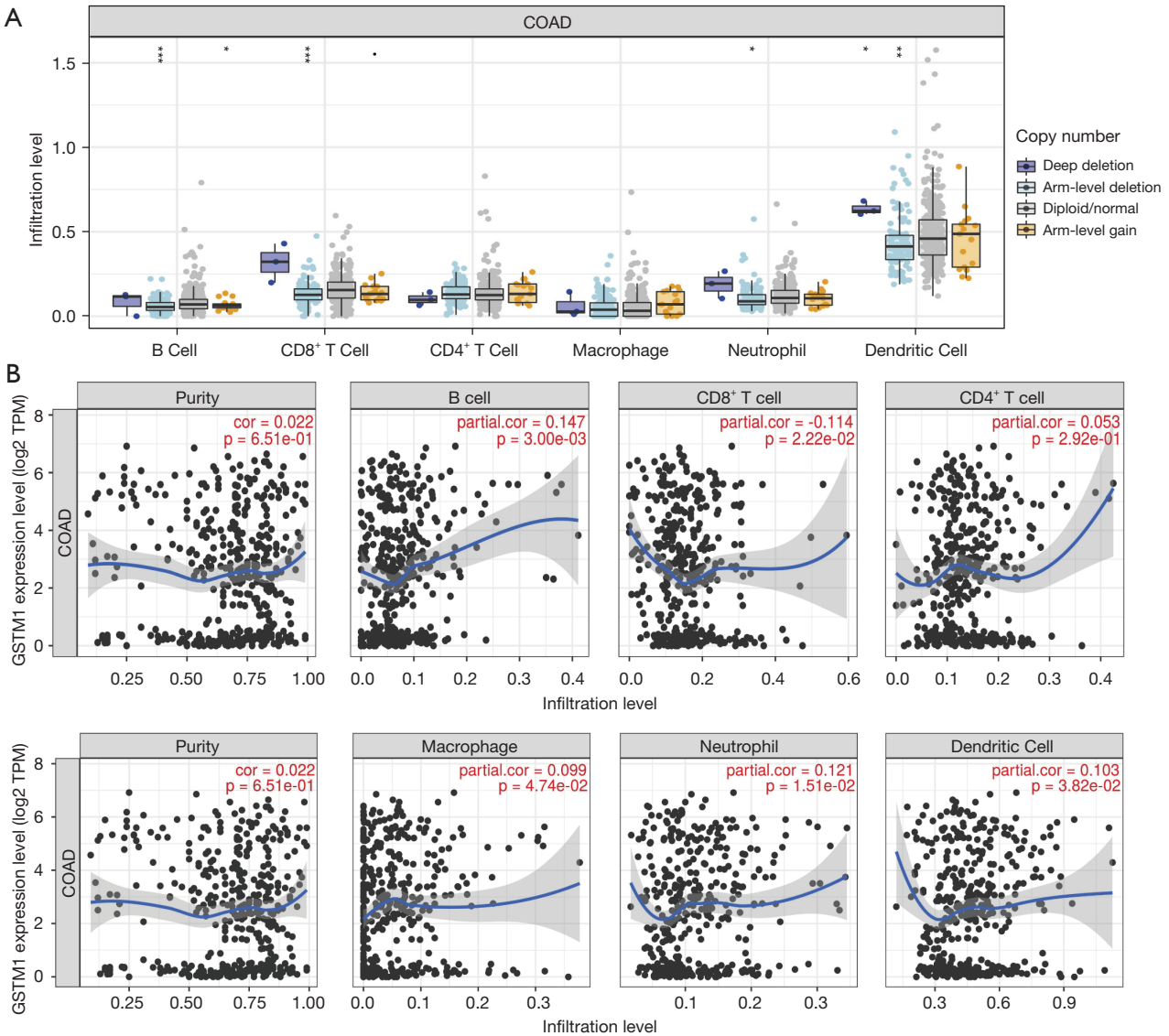


Figure 2 Correlations of *GSTM1* expression with immune cell infiltration level. (A) The association between *GSTM1* copy numbers and immune cell infiltration levels in COAD. (B) The correlation analysis of *GSTM1* expression levels and infiltration levels of dendritic cells, B cells, neutrophils, macrophages, CD8$^+$ T cell and CD4$^+$ T cell in COAD. *, P<0.05; **, P<0.01; ***, P<0.001. COAD, colon adenocarcinoma; *GSTM1*, glutathione S-transferase mu 1; TPM, transcript per million.

of *GSTM1* in COAD using the TCGA and HPA databases. As shown in *Figure 1B*, mRNA expression levels of *GSTM1* in COAD tissues (n=480) were remarkably lower than those in adjacent tissues (n=41). To carry out a comprehensive analysis of *GSTM1* protein expression, we used the UALCAN online tool. The results showed that the protein expression Z-value of *GSTM1* was decreased in COAD tissues (*Figure 1C*). In summary, these results provide evidence for the downregulation of *GSTM1* in COAD.

GSTM1 is correlated to the infiltration of immune cells

Further, we tried to determine whether *GSTM1* expression is related to immune cell infiltration in COAD. For this purpose, we conducted a correlation analysis through the TIMER "GENE" and "SCNA" modules. As shown in *Figure 2A*, the infiltration levels of B cells, CD8$^+$ T cells, neutrophils, and dendritic cells were decreased with the chromosome arm-level deletion. In addition, *GSTM1*

expression positively correlated with B cells (r=0.147, P=0.003), macrophages (r=0.099, P=0.047), neutrophils (r=0.121, P=0.015), and dendritic cells (r=0.103, P=0.038) (*Figure 2B*).

GSTM1 expression is related to immunomodulators

We assessed the relationship between *GSTM1* expression and immunomodulators based on the TISIDB baseline. Our results identified 23 immunostimulators and 13 immunoinhibitors that were positively or negatively associated with *GSTM1* expression in COAD (*Figure 3*). Based on these 36 *GSTM1*-associated immunomodulators, we constructed a PPI network using the STRING database. A strong correlation (r=0.7) of the 36 *GSTM1*-associated immunomodulators is shown in *Figure 4A*. At the same time, a pathway enrichment analysis for the 36 *GSTM1*-associated immunomodulators was performed, and our results demonstrated that some crucial related pathways, including T cell activation, regulation of T cell activation, and regulation of lymphocyte activation, were related to *GSTM1*-mediated immune events (*Figure 4B*).

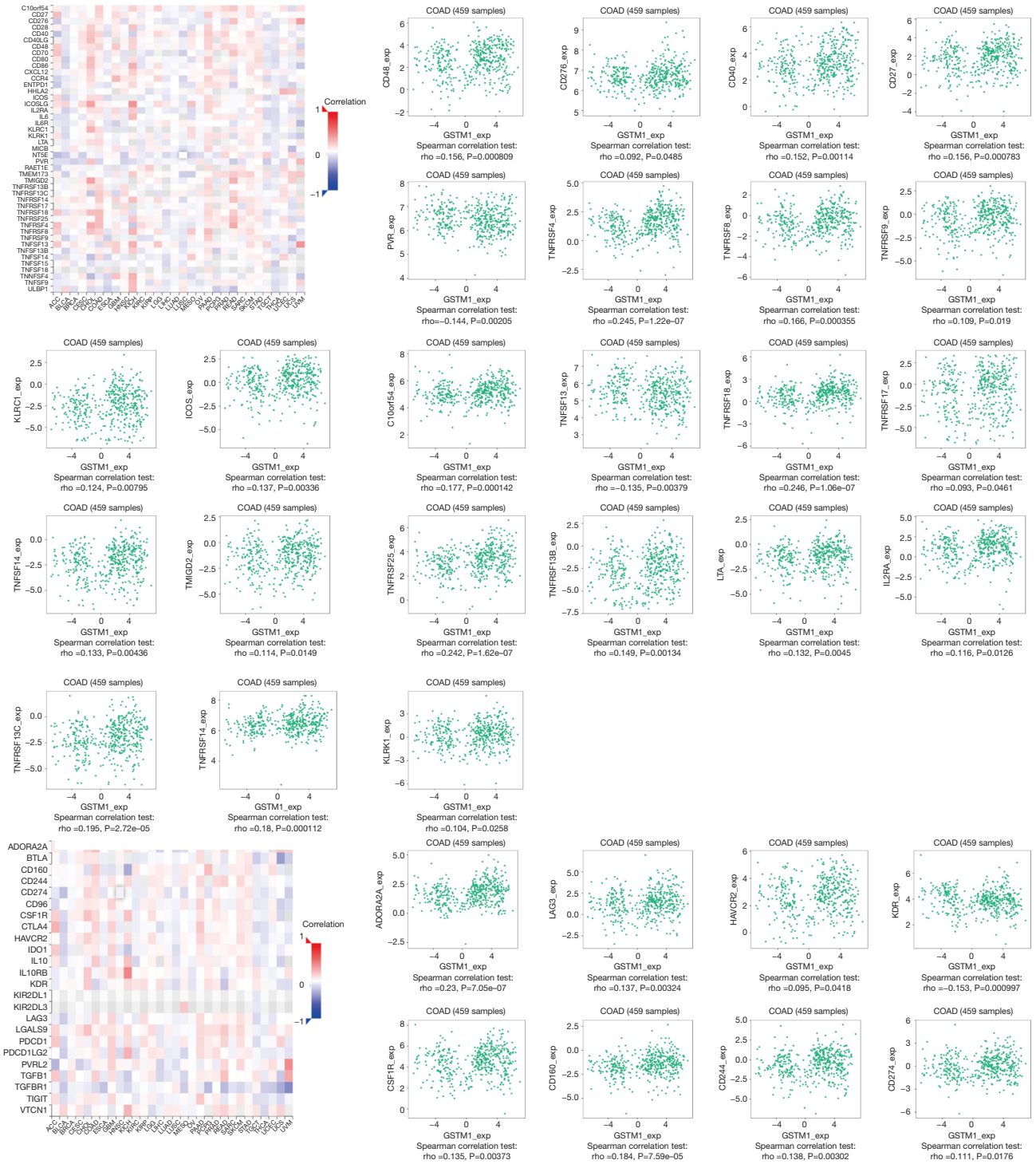
Establishment of a GSTM1-related immune prognostic risk model

To study the prognostic value of *GSTM1*-associated immunomodulators in COAD, we included the 36 *GSTM1*-associated immunomodulators in a stepwise multivariate Cox regression analysis and obtained an optimal 2-gene immune-related prognostic signature (*TNFRSF13C* and *TNFRSF25*) in TCGA-COAD patients (*Figure 5A*). In this prognostic risk model, the risk score for each patient can be calculated following the proposed formula: *TNFRSF13C* expression level × its coefficient (0.466) + *TNFRSF25* expression level × its coefficient (0.253). The TCGA-COAD patients were assigned into high- or low-risk groups based on the median value of the risk score. Afterward, we performed a Kaplan-Meier survival analysis based on the log-rank test; the results showed that the OS of high-risk patients was worse than that of low-risk patients (P=0.023) (*Figure 5B*). Next, we used the time-dependent ROC curve to assess the prediction accuracy of the risk model, and the area under the curve (AUC) values of the risk score and stage were 0.496 and 0.675, respectively. The AUC value was 0.747 when the risk score was combined with stage, suggesting a better prediction performance (*Figure 5C*). Then, we ranked each patient's risk score in ascending order, and the risk distribution map was plotted. As shown in *Figure 5D*, the expression of the high-risk genes *TNFRSF13C* and *TNFRSF25* were upregulated with the increasing risk score, and fewer deaths in the low-risk group were observed. In addition, using univariate and multivariate Cox regression, the risk score was identified as an independent prognostic factor for TCGA-COAD patients when adjusted for age and stage [hazard ratio (HR) =2.068, 95% confidence interval (CI): 1.628–2.626, P<0.001] (*Figure 5E*).

Finally, we constructed a prognostic nomogram by integrating the risk scores with other key clinical features, such as age and grade. Our constructed nomogram is a good tool for an accurate clinical prognostic assessment with a concordance index (C-index) value of 0.583 (*Figure 6A*). Moreover, the calibration curve showed that the probability predicted by the nomogram (gray line) had a good overlap with the ideal reference line (red line) for 3- and 5-year survival rates and especially for the 1-year survival rate (*Figure 6B*).

Discussion

In this study, we used different databases to study the expression differences of the *GSTM1* gene in cancer and normal tissues. We showed that expression of the *GSTM1* gene in gastrointestinal tumors such as colon cancer, gastric adenocarcinoma, prostate cancer, thyroid cancer, and endometrial cancer was low compared to that in normal tissues. In addition, by further studying the mRNA dataset and protein expression of colon cancer, we found that the expression of the *GSTM1* gene was significantly downregulated in colon cancer. Another major finding of this study is that *GSTM1* expression in COAD was related to the infiltration of a variety of immune cells. The TIMER analysis showed that the decreased copy number of *GSTM1* may lead to a decrease in B cells and that *GSTM1* expression was positively correlated with B cell infiltration. In addition, we constructed two *GSTM1*-related immunomodulator risk prognostic models (*TNFRSF13C* and *TNFRSF25*), in which *TNFRSF13C* is a member of the tumor necrosis factor (*TNF*) receptor superfamily. It has been reported that *TNFRSF13C* is also involved in the development of B lymphocytes and the survival of mature B cells (41-43). Moreover, *TNFRSF13C* is an attractive target for B-cell lymphoma (44). The evidence further emphasizes the importance of *GSTM1*-associated B cell-mediated immunity in COAD. Additionally, our findings displayed a positive correlation between *GSTM1* expression and macrophage



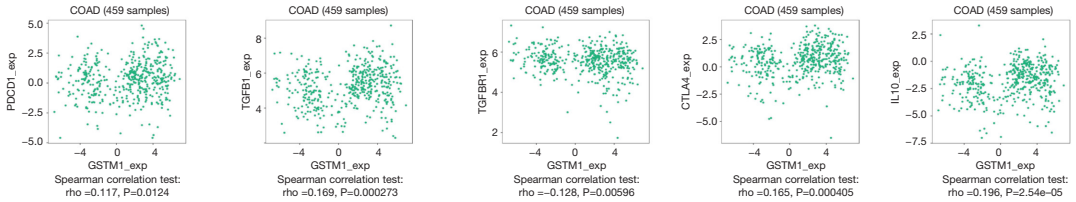


Figure 3 Correlations between *GSTM1* expression levels and immunomodulators. The heatmap and dot plots indicate that immunomodulators are significantly associated with *GSTM1* expression levels in COAD. Upper plot: immunostimulators; lower plot: immunoinhibitors. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; GSTM1, glutathione S-transferase mu 1; HNSC, head and neck squamous cell carcinoma; KIRP, kidney renal papillary cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, prostate adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

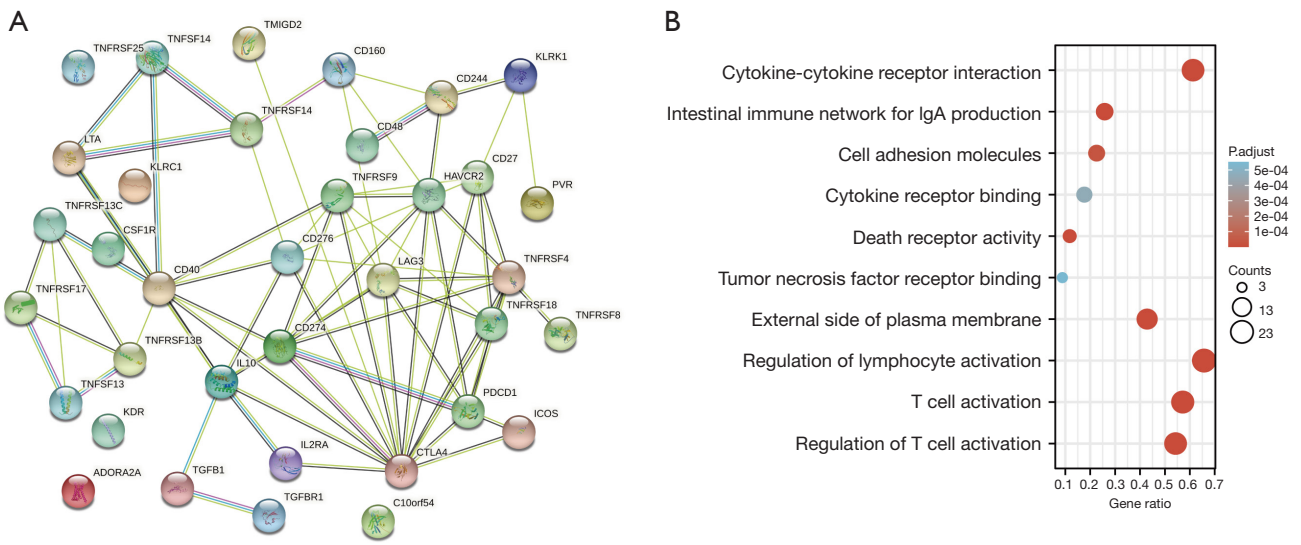


Figure 4 Analysis of *GSTM1*-associated immunomodulators. (A) The PPI network of 36 *GSTM1*-associated immunomodulators. (B) The functional enrichment analyses for the 36 *GSTM1*-related immunomodulators. *GSTM1*, glutathione S-transferase mu 1; PPI, protein-protein interaction.

infiltration. It has been well established that macrophages are the key components involved in inflammatory and tumor immune responses (45-47). Furthermore, research on therapy for tumor-associated macrophages suggests that various components of macrophages may become targets for new tumor treatments (48).

In addition, our study demonstrated that *GSTM1*-associated immunomodulators are involved in several crucial immune processes, including T cell activation, regulation of T cells, and lymphocyte activation. Lymphocytes are important immunocompetent cells in the immune system (49). The signal transduction and molecular basis of their activation process are extremely complex, and these immune processes have been considered important hallmarks for the prevention and control of cancer (50). Especially, T cell activation is a

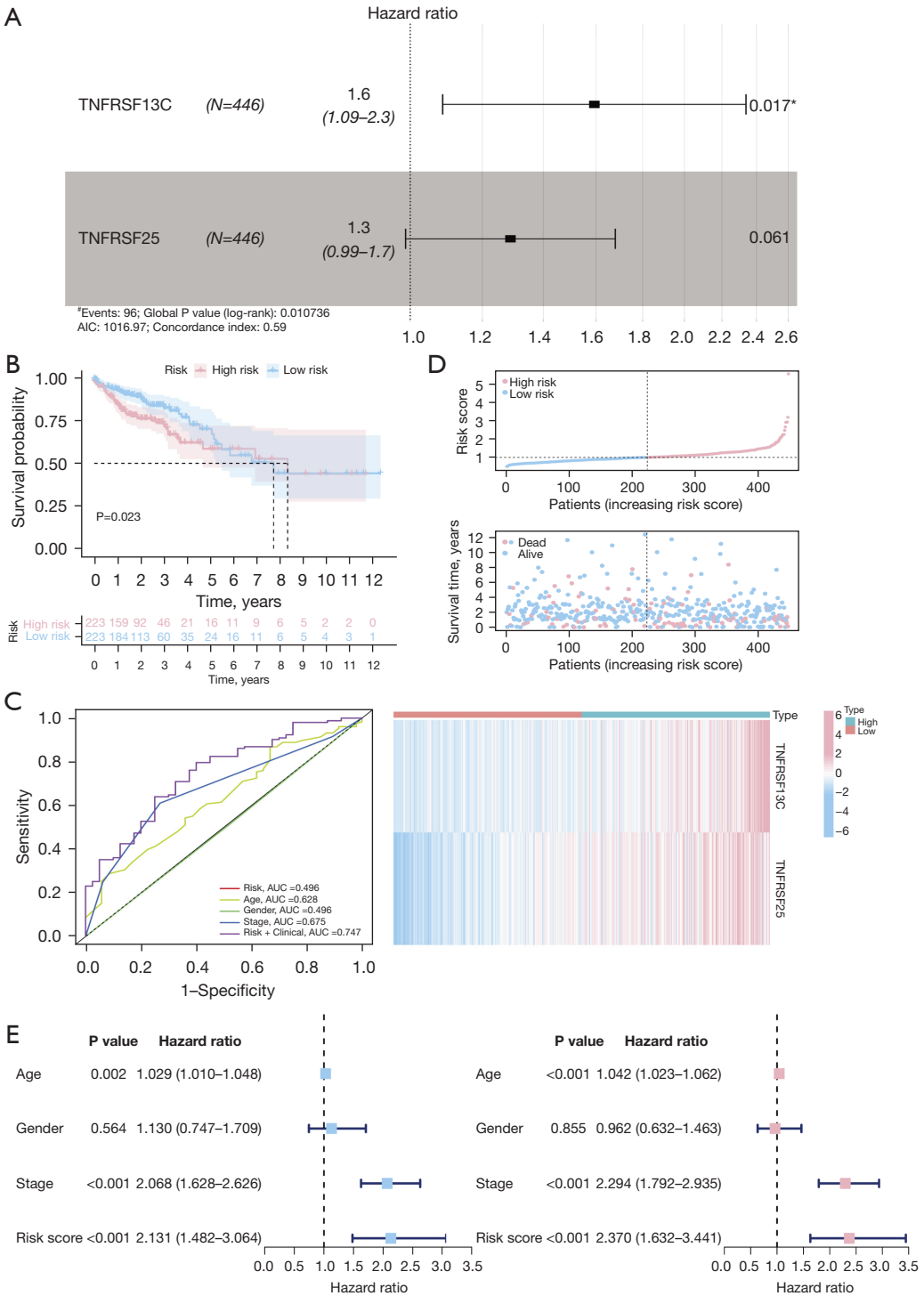


Figure 5 Construction of a 2-gene prognostic risk signature and the prognostic value of the risk score in TCGA-COAD patients. (A) The forest plot displaying the hazard ratios of genes integrated into the prognostic signature in TCGA-COAD patients. (B) The Kaplan-Meier survival analysis for patients with a high or low risk based on the risk score cutoff. (C) The time-dependent ROC curve for the risk score in the TCGA-COAD patients. (D) The distribution of patient risk scores, survival status, and expression patterns of hazard genes in TCGA-COAD patients. (E) The univariate and multivariate Cox regression analyses for the risk score in TCGA-COAD patients. *, P<0.05. AIC, Akaike information criterion; AUC, area under the curve; COAD, colon adenocarcinoma; TCGA, The Cancer Genome Atlas.

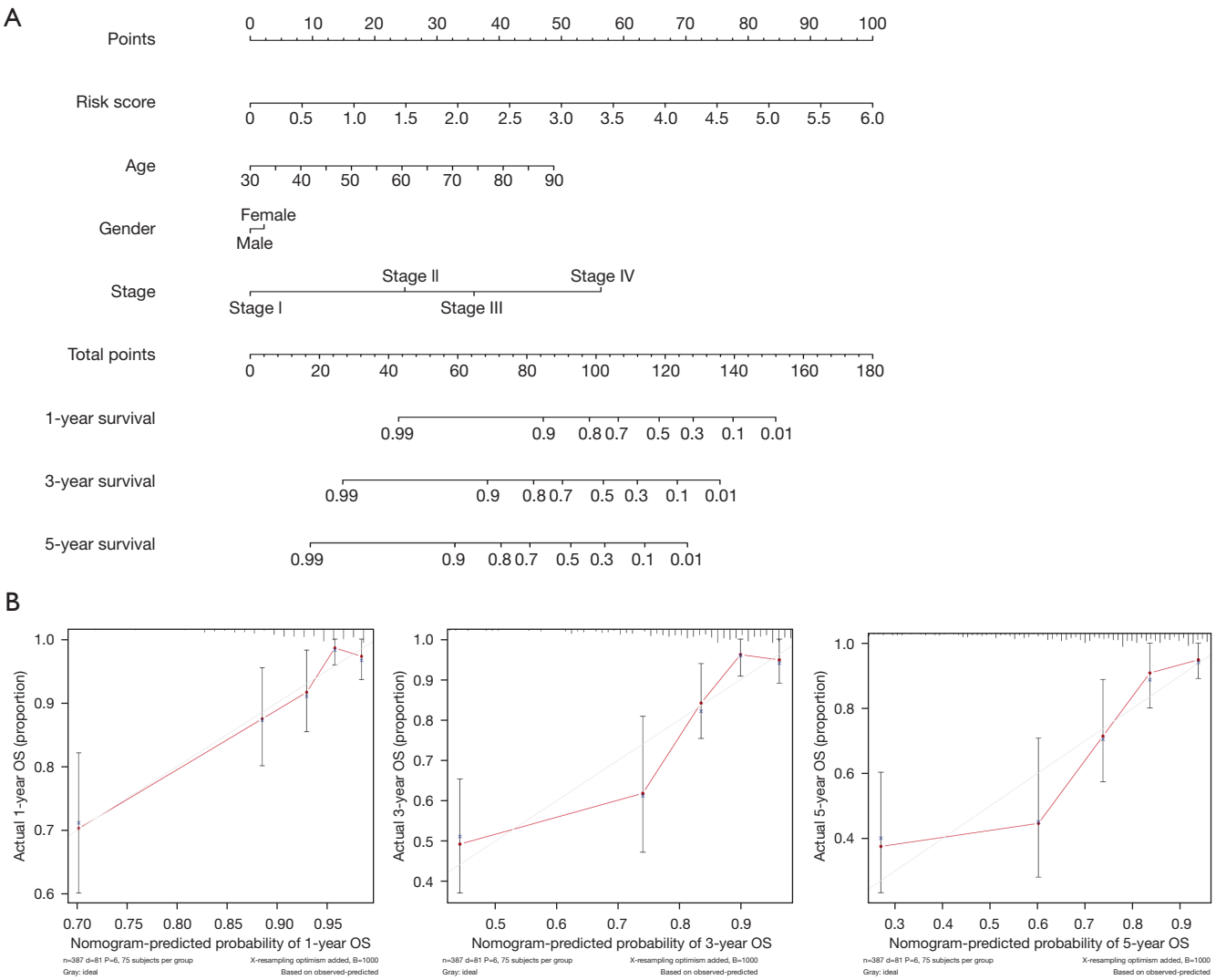


Figure 6 Establishment of the prognostic nomogram in TCGA-COAD patients. (A) A nomogram for predicting 1-, 3-, and 5-year survival rates for individual COAD patients. (B) The calibration curves of 1-, 3-, and 5-year survival in the TCGA-COAD patients. Red line: nomogram-predicted survival curve. Gray line: ideal survival reference curve. COAD, colon adenocarcinoma; OS, overall survival; TCGA, The Cancer Genome Atlas.

key and indispensable step for the anti-tumor immune response, and the effect mechanism of *PD-L1* is also based on T cell activation (40). Moreover, *PD-L1* can bind to the programmed cell death protein 1 of T cells, resulting in the inhibition of T-cell activity and impairment of anticancer T-cell immunity (51). Indeed, we also observed that the expression level of *GSTM1* in patients receiving anti *PD-L1* treatment and with response were higher than that those without response when investigating the response of *GSTM1* and immunotherapy (Figure S1). Based on the effect of *GSTM1* on immune cells and immune-related processes, we conclude that *GSTM1* might be a therapeutic target in COAD.

In recent years, several immune-related gene signatures have been identified and used for the prognosis prediction of colon cancer (52). For instance, Li *et al.* constructed a ten-gene immune-related signature that reflected the immune microenvironment and survival rates in colon cancer (53). Zhang *et al.* reported an immune-paired gene signature based on several public databases, which showed an independent prognostic role in colon cancer (54). We identified a two-gene immune-related signature

(*TNFRSF13C* and *TNFRSF25*) and established an immune-related prognostic risk model based on *GSTM1*-associated immunomodulators following a stepwise multivariate Cox regression analysis. To further study the clinical value of the immune-related prognostic risk model, we evaluated the relationship between the model and the OS of patients. We demonstrated that patients with high-risk scores had a worse prognosis. Multivariate Cox analysis further confirmed that the risk score of the model was an independent predictor for COAD patients. In addition, we combined the risk score with other key clinical features to construct a prognostic nomogram, which quantitatively predicts the individual risk of COAD. Moreover, the 1-year survival probability calibration curve demonstrated a good agreement between the survival probability predicted by the nomogram and the actually observed survival probability. These findings indicate that *GSTM1*-related 2 immunomodulators may be a good prognostic hallmark for COAD patients.

Conclusions

The combination of *GSTM1* and immune cell infiltration assessments is expected to allow for a more accurate evaluation of prognosis. However, the above results are bioinformatics analyses based on public data, which is a limitation of this study. It is necessary to carry out further experiments for verification. Our research provides evidence that *GSTM1* is closely correlated to tumor immunity. Furthermore, our identified *GSTM1*-related risk signature is a potential novel prognostic biomarker for prognosis in colon cancer.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1060/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1060/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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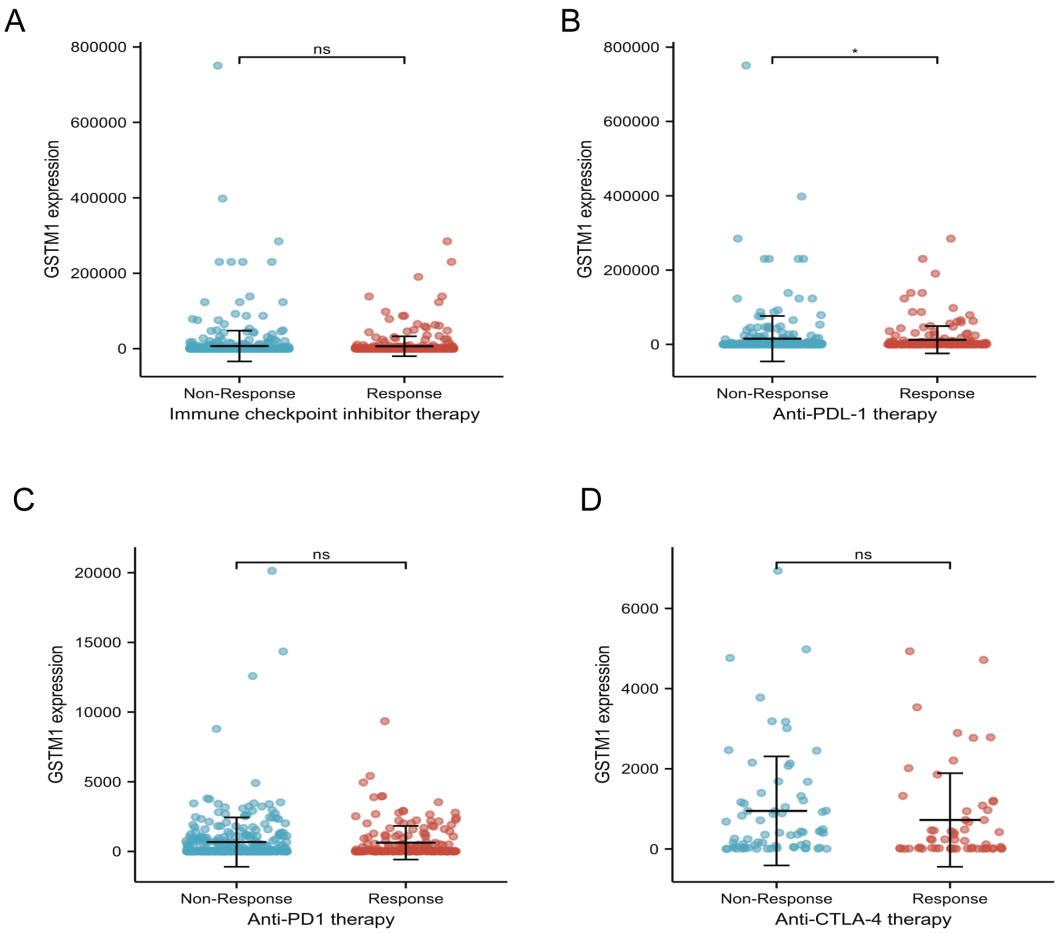

Figure S1 Immunotherapy response and GSMT1 expression. (A) GSTM1 expression level in immunotherapy responsive and non-responsive groups. (B-D) The expression of GSTM1 in three specific immunotherapies, including anti-PD-1, anti-PD-L1 and anti-CTLA-4 responsive and non-responsive groups. *, P<0.05. ns, not significant; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; GSTM1, glutathione S-transferase mu 1; PD-1, programmed death 1; PD-L1, programmed cell death-ligand 1.

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