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RESEARCH ARTICLE

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The cGAS-STING-related signature affects the prognosis of colorectal cancer through its regulation of multiple immune cells

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Abstract

The cGAS-STING signaling pathway has emerged as a critical player in the immune response against cancer, including colorectal adenocarcinoma (COAD). Understanding the impact of this pathway on COAD at multiple omics levels is crucial for advancing cancer immunotherapy and precision medicine. This study aimed to investigate the relationship between cGAS-STING-related genes and COAD, analyzing gene mutations, copy number variations, DNA methylation, and gene expression to uncover the pathway's influence on COAD prognosis. Utilizing multi-omics sequencing data from TCGA and GEO databases, key core genes in the cGAS-STING pathway were identified and further validated through PCR and Western blot analysis. Mutations and copy number variations in the CASP8 and RIPK1 genes, differential DNA methylation patterns, and mRNA expression levels of specific genes were assessed to determine their impact on COAD prognosis. Validation through tissue samples highlighted NLRC3, CASP1, AIM2, and CXCL10 as core genes in the cGAS-STING pathway. Our findings demonstrate that mutations and copy number variations in CASP8 and RIPK1, differential DNA methylation patterns, and altered gene expression levels significantly influence the prognosis of COAD. The identification of core genes in the cGAS-STING pathway, particularly NLRC3, CASP1, AIM2, and CXCL10, has led to the development of a prognostic model predicting poor tumor outcomes through immune cell infiltration. This study provides valuable insights into the mechanisms of the cGAS-STING pathway in COAD and offers potential directions for future research in cancer immunotherapy and precision medicine.

KEYWORDS

cGAS-STING, colorectal adenocarcinoma, multiple immune cells, prognosis

Yunlong Li, Xunliang Jiang and Hui Cao contributed equally.

1 | INTRODUCTION

The cGAS-STING signaling pathway is crucial for immune response as it detects abnormal DNA, such as those present in viruses and bacteria, and promotes interferon production by activating the STING-connected Interferon regulatory Factor 3 (IRF3) and nuclear factor kappa-B (NF-κB) pathways, leading to an increased immune response against pathogens in normal cells.[1–4] In tumors, cGAS can detect abnormal DNA resulting from DNA damage and chromosome rearrangement, leading to the activation of the cGAS-STING pathway[5–7] However, tumor cells can evade immune system attacks and resist activation of the cGAS-STING pathway through various mechanisms, thereby reducing the therapeutic effect in some types of tumors. This pathway is also closely linked to immunotherapy, where regulating its activity can improve the effectiveness of immunotherapy for specific cancers. For example, cGAS agonists can enhance the function of T cells and improve the anti-tumor immune response. On the other hand, inhibitors can be used to promote the growth of tumor cells that have already activated the cGAS-STING pathway and reduce attacks from the host's immune system.[8–10] These findings highlight the importance of the cGAS-STING pathway in anti-tumor immunotherapy research and its potential for improving cancer treatment.

The cGAS-STING signaling pathway is a critical factor in the development of colorectal cancer, and scientists are actively researching its role. Studies have shown that inactivation of the trans-isomerase ten-eleven translocation (TET) gene can lead to changes in DNA methylation, impacting the immune system. Inflammation and microbiota also contribute to the activation of the pathway in the intestine, which is a crucial factor in the development of colorectal cancer.[7,11] Current research on the cGAS-STING signaling pathway in colorectal cancer has found that loss of TET can promote cancer cell growth and metastasis, whereas STING agonists can enhance the immune response and treat colorectal cancer. The discoveries regarding the role of the cGAS-STING signaling pathway in colorectal cancer could have significant therapeutic benefits.[12,13]

Our study aims to address a gap in research on the relationship between the cGAS-STING signaling pathway and colorectal adenocarcinoma (COAD). Currently, existing studies only focus on downstream mechanisms of a single gene, while we aim to understand the overall relationship between the cGAS-STING signaling pathway and COAD from a multi-omics perspective. To achieve this, we analyzed the relationship between cGAS-STING-related genes and COAD using gene mutation, copy number variation, DNA methylation, and gene expression data from the Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. We identified core genes that play a crucial role in the cGAS-STING signaling pathway in COAD for validation. Additionally, we constructed a cGAS-STING-related prognostic model to determine whether these genes can be used as prognostic indicators for COAD. Overall, our study provides valuable insights into the relationship between the cGAS-STING signaling pathway and COAD at multiple omics levels, which may have clinical implications for the prognosis and treatment of COAD.

2 | MATERIALS AND METHODS

2.1 | Data acquisition

To conduct a comprehensive analysis of the impact of cGAS-STING on COAD, we acquired multiple datasets, including RNA-seq, Whole Genome Sequencing (WGS), DNA methylation sequencing, and clinical parameter data from TCGA-COAD using UCSC XENA. Additionally, we utilized the GEO database, which contains 693 colon cancer sequencing data and 20 normal samples, to conduct differential expression and prognosis analyses of cGAS-STING-related genes. The cGAS-STING-related prognostic model was validated using external datasets.

2.2 | Construction of prognostic model and analysis of clinical characteristics

In this study, LASSO regression was utilized to identify cGAS-STING genes that have an impact on COAD prognosis. The selection of the cGAS-STING genes for constructing the prognostic model was based on the minimum lambda. The Kaplan–Meier algorithm was utilized to analyze the relationship between the cGAS-STING model and COAD overall survival (OS), progression-free interval (PFI), and disease-specific survival (DSS). The cGAS-STING prognostic model was further divided into high and low expression groups based on the best grouping. Additionally, the relationship between the cGAS-STING model and other COAD clinical parameters was analyzed, including age, sex, radiotherapy, and chemotherapy status, TNM stage, tumor recurrence status, and the cGAS-STING model, based on TCGA-COAD clinical information.

2.3 | Functional analysis of prognostic models

To analyze the RNA-seq data based on the cGAS-STING prognostic model, we utilized the “limma” package and

applied criteria of |Log2 fold change| >1.0 and adjusted $p < .05$ to identify differentially expressed genes. We further investigated the functions that might be affected by the prognostic model. To this end, we utilized the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, which contains various gene reaction pathways. We performed KEGG enrichment analysis on the differentially expressed genes using the "clusterProfiler" package and Gene Set Enrichment Analysis (GSEA) algorithm. Additionally, we evaluated the immune infiltration score and tumor feature pathway score of COAD patients using the Gene set variation analysis (GSVA) algorithm. This allowed us to analyze the impact of the cGAS-STING model on immune cells and tumor feature pathways.

2.4 | Human tissue sample verification

We collected 22 tissue samples of colorectal cancer patients from The First Affiliated Hospital of the Military Medical University of the PLA Air Force to verify whether there was differential expression of the core genes. We used real-time quantitative Polymerase Chain Reaction (PCR) and western blot to verify the expression of the four genes at both mRNA and protein levels. GAPDH and β-actin were used as internal reference genes for expression normalization. The specific primers and protein antibody information for the four genes can be found in Table S1.

2.5 | Apoptosis assay

Apoptosis was evaluated 72 h post-transfection. Cells were detached using trypsin without Ethylenediaminetetraacetic acid (EDTA) and subsequently centrifuged at 300*g* for 5 min at 4°C to facilitate collection. The resulting cell pellet was washed twice with phosphate buffer saline (PBS). After discarding the supernatant, the cells were resuspended in 100 μL of 1× binding buffer. For staining, 5 μL of Annexin V-Fluorescein isothiocyanate (FITC) and 10 μL of PI staining solution were added to the suspension, followed by gentle mixing. The cell suspension was incubated in the dark at room temperature for 10–15 min. Following incubation, 400 μL of diluted 1× binding buffer was added to the samples, which were then mixed thoroughly before flow cytometric analysis.

2.6 | Wound-healing assay

Cells were transfected with plasmids for 24 h and subsequently scratched using a pipette tip to create a wound. The monolayer was washed once with PBS and then replenished with complete culture medium. Initial images of the wound were captured immediately after washing, and the cells were incubated for an additional 72 h to facilitate migration and proliferation into the wounded area.

2.7 | Statistical analysis

The data were analyzed and visualized using R software version 4.1.2, and the measurement data were reported as the mean along with the standard deviation. The Wilcoxon rank sum test was applied to determine any differences between two groups, while Kaplan–Meier log-rank analysis was employed to assess survival differences between grouped patients. The influence of cGAS-STING on COAD prognosis was evaluated using the Cox regression model. Results with a *p*-value less than .05 were deemed statistically significant.

3 | RESULTS

3.1 | Data and gene selection

Based on the PathCards database, we collected a total of 103 cGAS-STING-related genes. To understand the basic functions among these 103 genes, we first conducted protein interaction analysis. As shown in Figure 1A, most of the genes have certain interaction relationships. Furthermore, enrichment analysis of the above genes revealed that the 103 genes are mainly related to the cGMP-PKG signaling pathway and the Toll signaling pathway in the KEGG pathway enrichment analysis, which also confirms that the genes we collected are correct. GO analysis showed that the IL-17-mediated signaling pathway is related (Figure 1B).

Using UCSC XENA to download RNA-seq, whole genome sequencing, methylation sequencing data, and clinical parameter data from TCGA-COAD for a comprehensive analysis of the impact of cGAS-STING-related diseases on COAD. In the subsequent analysis of clinical parameters, we classified N staging into N0 and N1+. T staging was divided into T1–2 and T3–4. Other basic clinical parameters were the same as those provided in Table 1.

3.2 | cGAS-STING gene mutation affects the prognosis of COAD

We utilized maftools to investigate the gene mutation data of TCGA-COAD. Our analysis indicated that

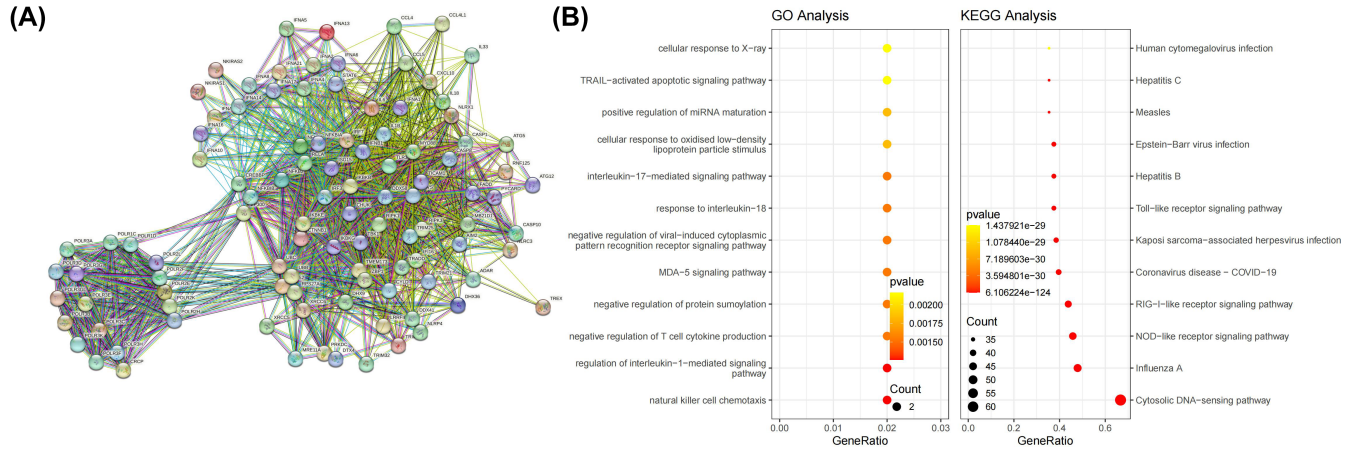

FIGURE 1 Basic information analysis of cGAS-STING-related genes. (A) Protein interaction analysis between cGAS-STING-related genes; (B) functional enrichment analysis of cGAS-STING-related genes in GO and KEGG. The size of the bubbles in the figure represents the number of genes included, and the darker the color, the more significant the result.

99 cGAS-STING-related genes had gene mutations, and most of these mutations were missense mutations (Figure 2A). Among all the mutated genes, the PRKDC gene had the highest frequency of occurrence, with mutations present in 11% of the patients. However, most of these mutations were unique to each patient, and no hotspot mutations were observed. CREBBP was second on the list, with mutations present in 10% of the patients and had a high-frequency mutational hotspot near the Bromo_cbp_like domain (Figure 2B). Further analysis of the top 10 genes with the highest mutation frequency revealed that multiple cGAS-STING gene mutations had co-occurrence relationships. The gene with the highest mutation frequency, PRKDC, had a co-occurrence with most of the genes (Figure 2C). Our analysis of COAD patients based on whether they had cGAS-STING mutations revealed that cGAS-STING gene mutations were correlated with Microsatellite Instability (MSI) and had a high frequency of mutation occurrence in the MSI-H distribution (Figure 2D). Finally, our analysis of the relationship between each gene mutation and tumor prognosis revealed that mutations in the CASP8 and RIPK1 genes were associated with a poor prognosis in COAD patients (Figure 2E).

3.3 | cGAS-STING gene copy number variation affects the prognosis of colon cancer

Using the copy number variation data of TCGA-COAD, we found a total of 99 genes with changes in copy number. The genes with the largest increase in copy number were ZBP1 (72.28%), POLR1D (60.08%), TAC1 (81.6%), and Interleukin-6 (IL6) (56.54%). On the other hand, RNF125 had the most significant decrease in copy number, accounting for 58.31% of all samples (Figure 3A).

We performed a prognosis analysis on the genes with copy number variations to observe whether cGAS-STING copy number changes affect the prognosis of COAD. After analysis, we found that the copy number changes of 31 genes affected the prognosis of COAD. Among them, CREBBP was a gene related to prognosis, and its copy number changes also affected the prognosis of COAD, with increases, no changes, and decreases in copy number each having an impact on tumor prognosis (Figure 3B). In addition to copy number increases leading to better prognoses, changes in the IFNA16 gene copy number also resulted in a worse prognosis, whether it increased or decreased (Figure 3C). Furthermore, we further analyzed the relationship between the above significant prognostic genes and clinical parameters of COAD. After analysis, we found that there was a relationship between CREBBP and N stage, M stage, and STAGE stage, with copy number increasing as the disease worsened (Figure 3D). In addition, COAD's MSI was also related to genes such as DDX4, CTNNB1, and CCL4 (Figure 3E).

3.4 | The cGAS-STING gene's DNA methylation has an impact on the prognosis of COAD

In this study, we focused on analyzing methylation sites in the promoter and enhancer regions of genes, since gene methylation predominantly affects gene expression through these sites. In instances where a gene promoter

TABLE 1 Basic clinical features of TCGA-colorectal adenocarcinoma.

Characteristic	$N = 551$[a]
Age	
≤60	159 (29%)
>60	387 (71%)
Unknown	5
Gender	
Female	262 (48%)
Male	284 (52%)
Unknown	5
Sample_type	
Metastatic	2 (0.4%)
Primary tumor	462 (84%)
Recurrent tumor	1 (0.2%)
Solid tissue normal	85 (15%)
Unknown	1
Pathologic_M	
M0	398 (84%)
M1	78 (16%)
Unknown	75
Pathologic_N	
N0	323 (59%)
N1+	223 (41%)
Unknown	5
Pathologic_T	
T1-2	101 (19%)
T3-4	444 (81%)
Unknown	6
Pathologic_stage	
Stages I and II	305 (57%)
Stages III and IV	230 (43%)
Unknown	16
Recurrence	102 (23%)
Unknown	108
MSI	
MSI-H	97 (18%)
MSI-L	97 (18%)
MSS	333 (63%)
Unknown	24
Pharmaceutical_therapy	43 (57%)
Unknown	476
Radiation_therapy	14 (17%)
Unknown	470
Total_mutation	113 (70, 170)
Unknown	360

[a]*n* (%); median (IQR).

region had multiple CG sites, we used the mean value to represent the average methylation rate of the gene. Our initial analyses focused on identifying differentially methylated genes between COAD and normal samples. A total of 41 methylation sites were identified as exhibiting variations between cancer and normal samples. Of these, 38 were situated in the promoter region and three were in the enhancer region (Figure 4A). It is noteworthy that these three enhancer sites, located on the IL6 gene, were found to be hypomethylated. Functional enrichment analysis of differentially methylated genes identified that the differential methylation sites were significantly associated with the TNF signaling pathway (Figure 4B). Furthermore, we conducted a prognosis analysis to explore the impact of cGAS-STING methylation on COAD prognosis. Our findings revealed that methylation of the promoter region of 37 genes affected the prognosis of COAD. Notably, two of these genes, CASP8 and RIPK1, were found to be affected by gene mutations and prognosis. Additionally, the methylation of Interleukin 18 (IL18), a gene encoding interleukin, also impacted the prognosis of COAD (Figure 4C). To investigate the relationship between the methylation of the promoter region of the identified 63 genes and clinical parameters of COAD, we further analyzed our findings. Our analysis revealed that the methylation of multiple genes, including CTNNB1, CCL5, and CASP8, was significantly associated with MSI. Interestingly, these genes exhibited high methylation levels in the MSI-H group (Figure 4D).

3.5 | cGAS-STING-related gene expression affects the prognosis and immune cell infiltration of COAD

To identify cGAS-STING genes that affect the occurrence of COAD, we utilized TCGA TPM expression data and performed differential expression analysis using the limma package. Our analysis identified 37 genes with differential expression (Figure 5A). We also performed a similar differential expression analysis on GEO data, which identified 28 differentially expressed genes (Figure 5B). From these datasets, we identified 20 stable differentially expressed genes common to both datasets (Figure 5C). Functional enrichment analysis of these differentially expressed genes revealed that they were predominantly associated with the cytosolic DNA-sensing pathway (Figure 5D). To explore the impact of cGAS-STING-related genes on COAD prognosis, we conducted a prognosis analysis using TCGA and GSE39582 datasets. We selected genes that had an impact on prognosis in both datasets and found a total of 38 prognostic genes (Figure 5E). KEGG pathway enrichment analysis of these

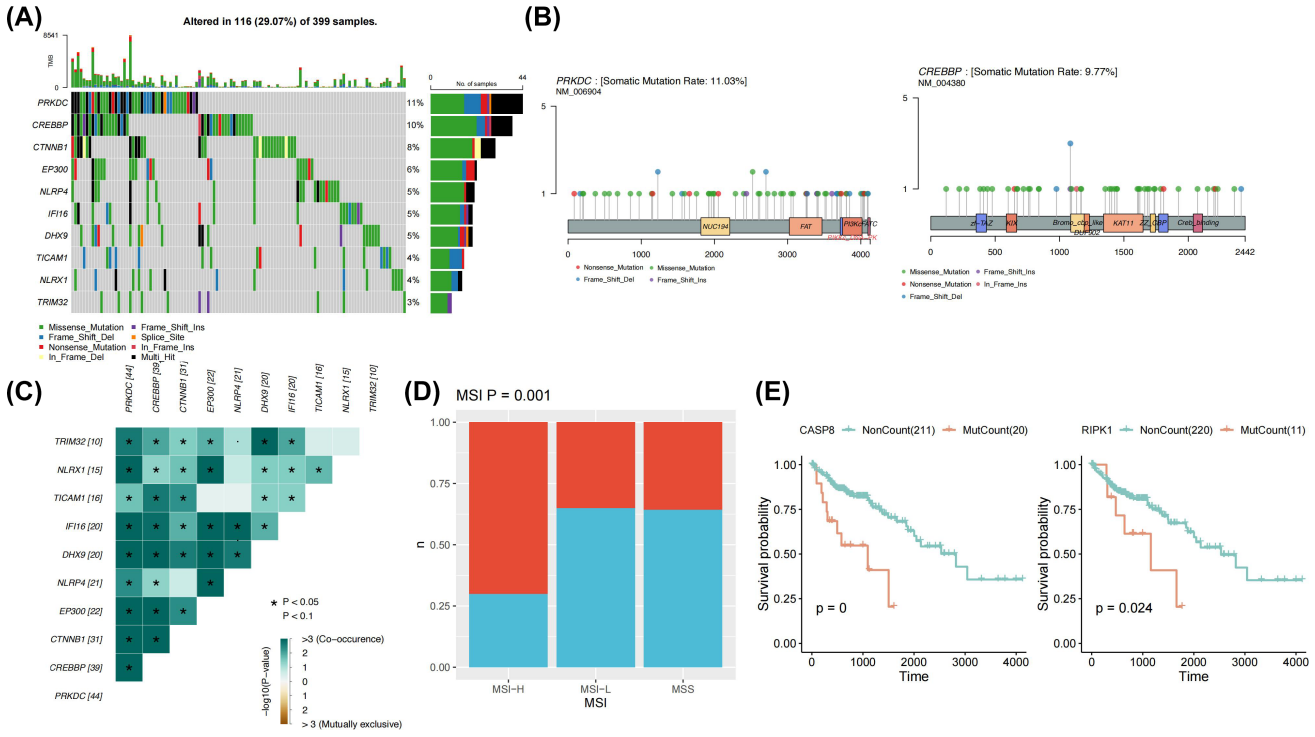

FIGURE 2 Relationship between cGAS-STING signaling pathway gene mutations and colorectal adenocarcinoma (COAD). (A) Waterfall plot of gene mutations related to the cGAS-STING signaling pathway; (B) specific locations of PRKDC and CREBBP gene mutations; (C) mutual occurrence/exclusion analysis of gene mutations in the cGAS-STING signaling pathway; (D) relationship between cGAS-STING signaling pathway mutations in COAD patients and MSI; (E) effect of CASP8 and RIPK1 gene mutations on the prognosis of COAD.*means p less than 0.05.

genes revealed that they were mainly associated with the Toll-like receptor signaling pathway (Figure 5F). Next, we integrated the four datasets and selected genes that had differential expression in both COAD incidence and prognosis. From this analysis, we identified four characteristic cGAS-STING genes of COAD: NLRC3, CASP1, CXCL10, and AIM2 (Figure 5G). We further analyzed the relationship between the four genes and clinical parameters of COAD and found that their expression levels were significantly associated with the Stage and M stage of COAD. Specifically, NLRC3 and AIM2 had low expression in the M1 stage, while CASP1 and CXCL10 had high expression in the M1 stage (Table 2). To understand the biological functions of the four characteristic genes, we conducted functional enrichment analysis. KEGG pathway enrichment analysis revealed that these genes were mainly associated with the IL-17 signaling pathway (Figure 5I). Additionally, the presence of immune cells is a crucial factor in the development and management of tumors. To gain a deeper understanding of the connection between key genes and immune cells, we utilized the ESTIMATE algorithm to assess the immune cell levels in each tumor sample. Subsequently, we examined the relationship between the four key genes and immune scores. Our analysis revealed a strong correlation between three of the genes and immune cells, while CASP1 showed a weaker correlation (Figure S1). To gain a deeper understanding of the specific immune cells associated with the four genes, the GSVA algorithm was utilized to assign a score to each cell. Our analysis revealed that CASP1 is not significantly linked to immune cells, whereas the remaining three genes are associated with a diverse range of immune cells (Figure 5J).

To delve deeper into the stable expression of core genes in colon cancer, we procured samples of colon cancer tissue and adjacent non-cancerous tissue from 22 patients affected by colorectal cancer. We used PCR and Western blotting (WB) techniques to confirm that the four core genes showed differential expression in both RNA and protein (Figure 6). Additionally, we scrutinized the association between the four core genes and clinical features such as age and gender. Post-analysis, it was established that there was no correlation between the four genes and clinical parameters.

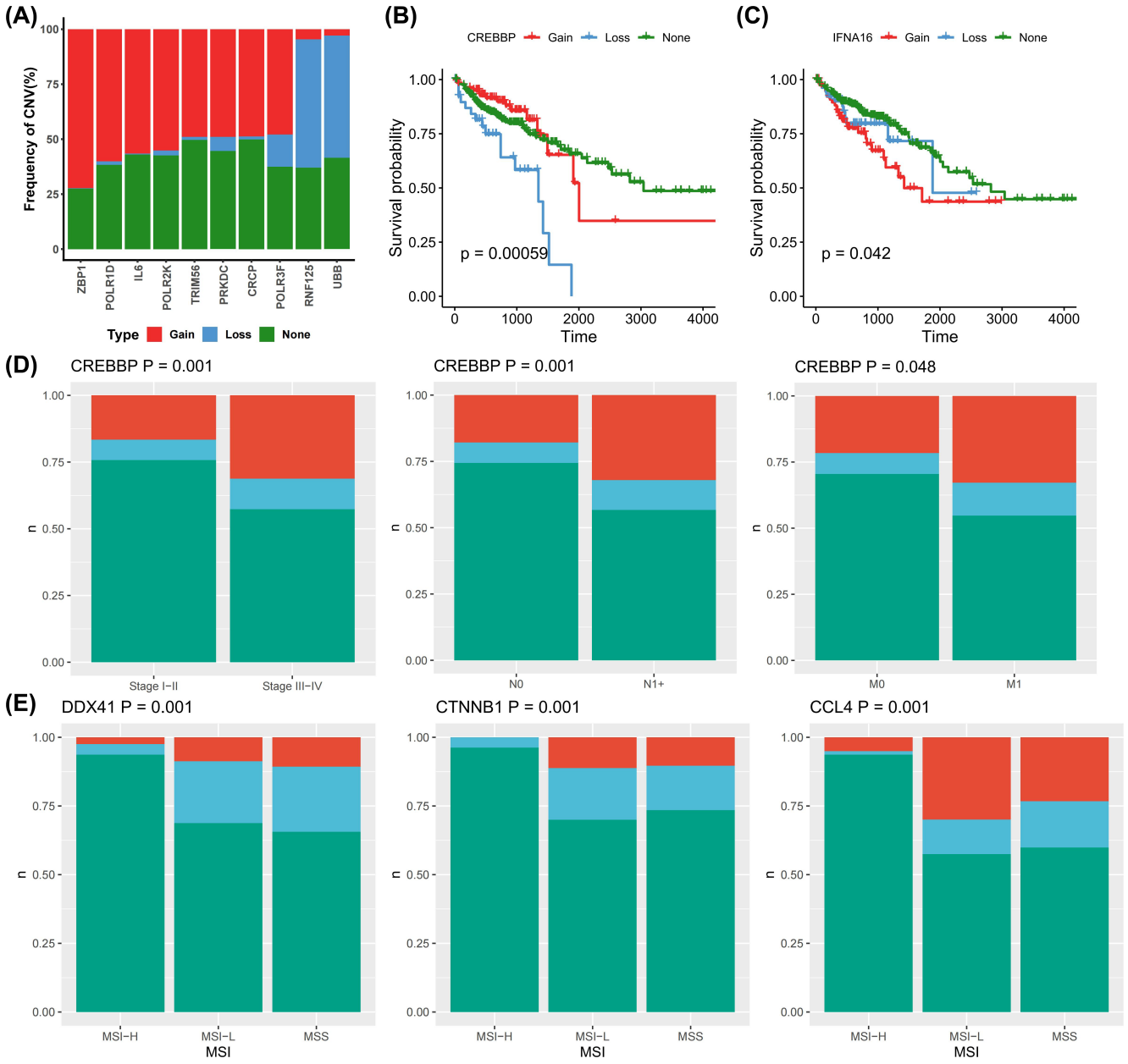

FIGURE 3 Relationship between cGAS-STING signaling pathway gene copy number changes and colorectal adenocarcinoma (COAD). (A) Top 10 genes ranked by copy number changes; (B) effect of CREBBP gene copy number changes on the prognosis of COAD patients; (C) survival analysis of IFNA16 gene copy number changes; (D) relationship between CREBBP and stage, N stage, and M stage; (E) relationship between MSI and gene copy number changes in COAD patients.

3.6 | DNA methylation and copy number variation affect the expression of key genes

Various factors, such as genetic mutations, DNA methylation levels, and copy number variations, can impact gene expression. Our previous analysis focused on the connection between cGAS-STING-related genes and COAD using a single omics approach. However, further investigation is needed to understand the interplay between multiple omics and gene expression omics. To identify potential regulatory factors that may influence the characteristic cGAS-STING genes, we analyzed gene mutations, methylation, and copy number variations.

3.6.1 | Gene mutations

Our analysis did not reveal any significant mutations in the four characteristic genes, suggesting that mutations are not a regulatory factor in controlling gene expression.

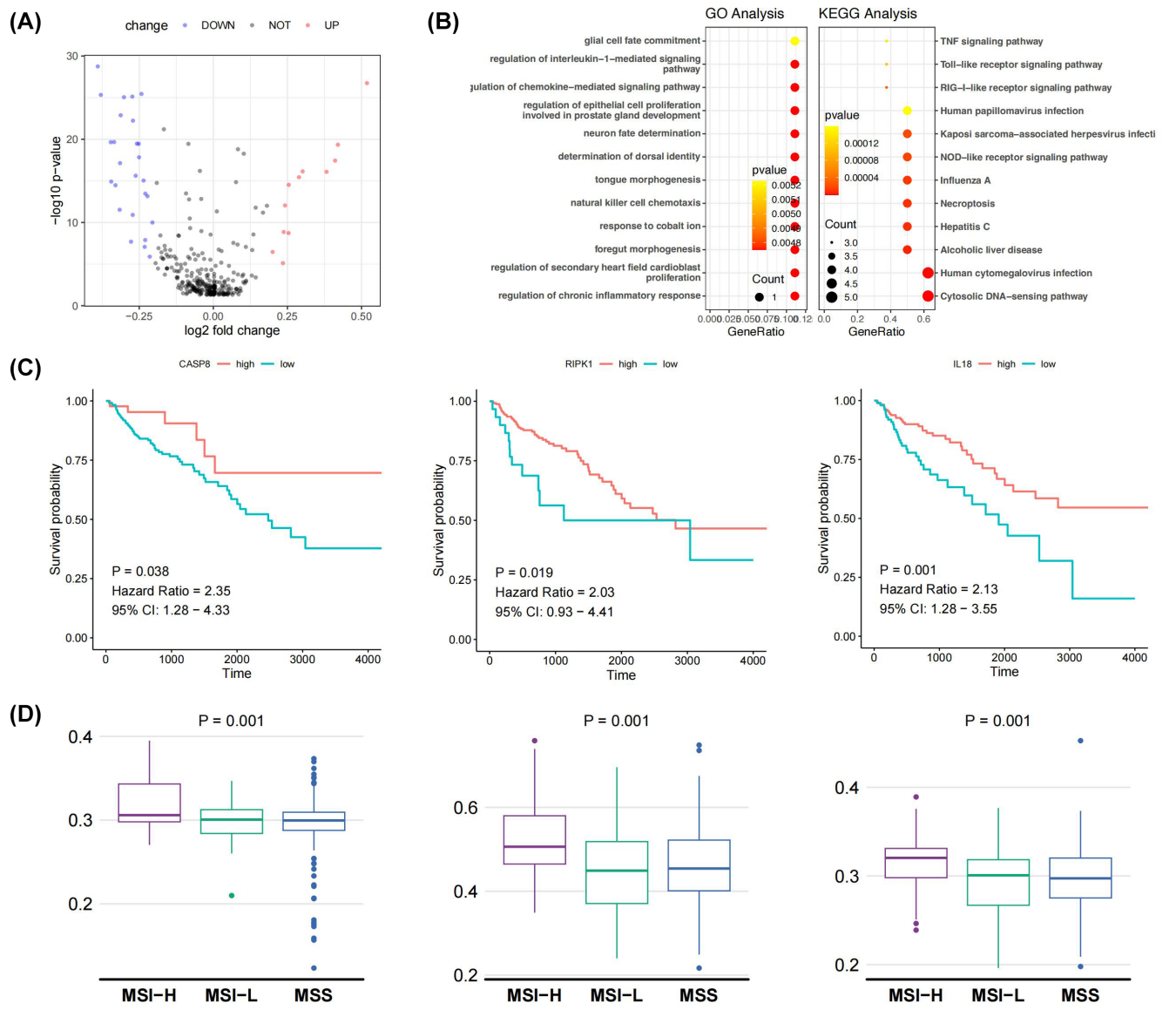

FIGURE 4 Relationship between cGAS-STING signaling pathway gene methylation and colorectal adenocarcinoma (COAD). (A) Volcano plot of differential methylation analysis; (B) functional enrichment analysis of differentially methylated genes in GO and KEGG. The size of the bubbles in the figure represents the number of genes included, and the darker the color, the more significant the result; (C) effect of gene methylation levels on the prognosis of COAD; (D) relationship between CTNNB1, CCL5, and CASP8 methylation and MSI.

3.6.2 | Methylation

Our analysis showed that NLRC3, CASP1, and AIM2 were subject to a certain degree of regulation by methylation. NLRC3 was negatively regulated by multiple methylation sites, while only one methylation site (cg07762961) was positively correlated with expression (Figure 7A). Similarly, we found that CASP1 expression was negatively regulated by methylation (Figure 7B). AIM2 was regulated by two methylation sites; cg11003133 was negatively correlated with AIM2 expression, while cg24659501 was positively correlated with AIM2 expression (Figure 7C).

3.6.3 | Copy number variations

Our analysis revealed that changes in gene copy number were significantly associated with the expression of CASP1 and AIM2. Interestingly, we found that changes in copy number were associated with increased gene expression (Figure 7D).

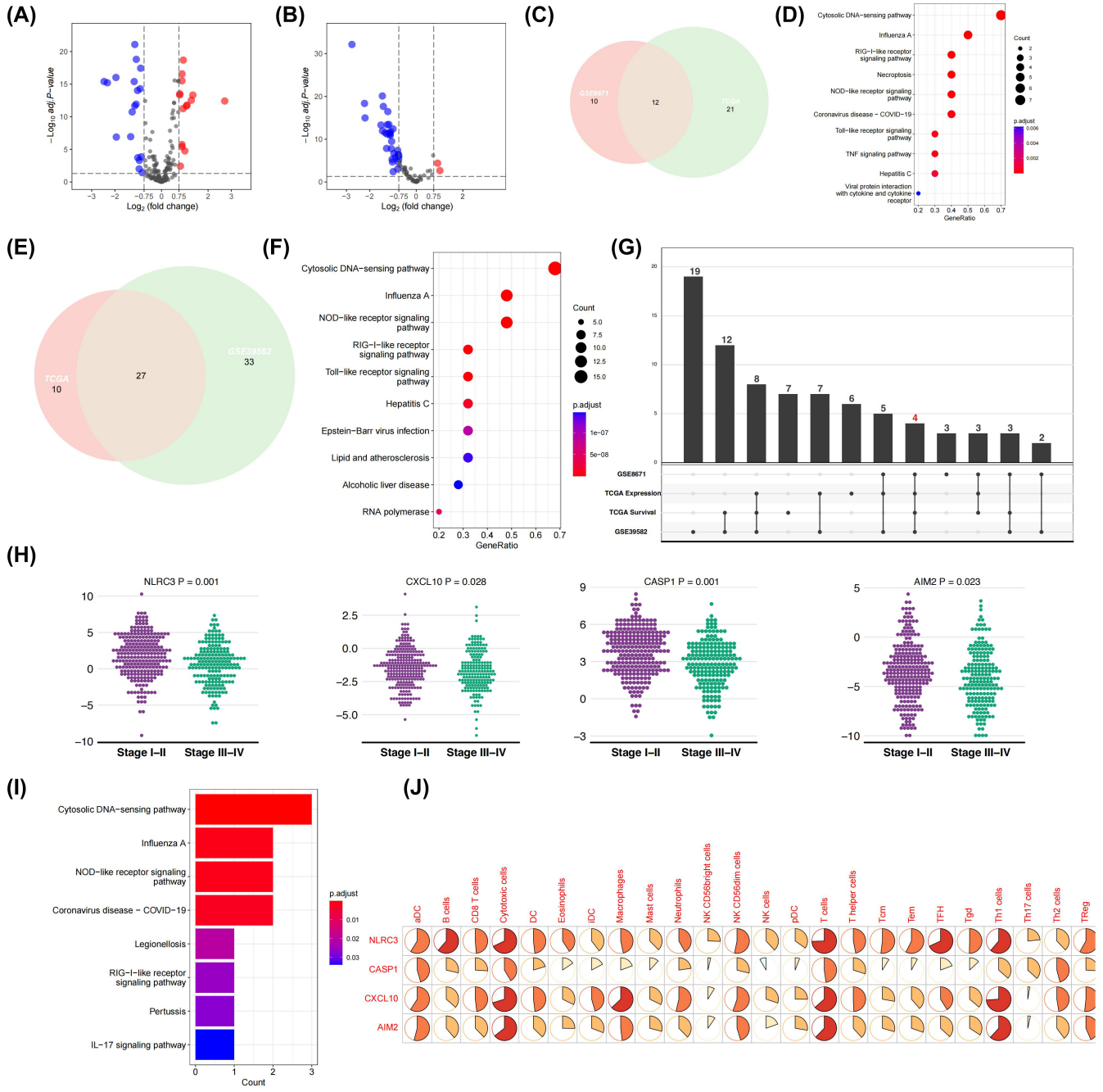

FIGURE 5 Relationship between mRNA expression of cGAS-STING signaling pathway genes and colorectal adenocarcinoma (COAD). (A) Volcano plot of differential expression analysis of TCGA-COAD; (B) volcano plot of differential expression analysis of GSE8671, where blue bubbles represent low-expression genes, red bubbles represent high-expression genes, and black bubbles represent genes without differential expression; (C) Venn diagram of cross-analysis of differential genes in TCGA-COAD and GSE8671; (D) functional enrichment analysis of stably differentially expressed genes; (E) Venn diagram of cross-analysis of significant prognostic genes in TCGA-COAD and GSE39582; (F) KEGG pathway enrichment analysis of significant prognostic genes; (G) upset plot of cross-analysis of TCGA-COAD, GSE8671, and GSE39582, where the upper part of the figure represents the genes from the combination of the four datasets, and the red labels represent the number of significant genes in the four datasets; (H), effect of core genes on clinical stage; (I) KEGG pathway enrichment analysis of the four core genes; (J) relationship between core genes and immune cell infiltration, where red represents positive correlation and blue represents negative correlation, and the larger the size of the pie chart, the more significant the correlation.

Characteristic	M0, $N = 333$[a]	M1, $N = 64$[a]	p-Value[b]
NLRC3	−1.41 (−2.35, −0.42)	−2.23 (−2.81, −1.40)	<.001
CASP1	3.74 (2.28, 5.02)	2.70 (1.41, 3.70)	<.001
CXCL10	1.69 (0.05, 3.80)	0.05 (−1.73, 1.78)	<.001
AIM2	−3.62 (−5.22, −1.65)	−4.86 (−6.34, −3.00)	.006

[a]Median (IQR).
[b]Wilcoxon rank sum test.

TABLE 2 Relationship between four key genes and M stage.

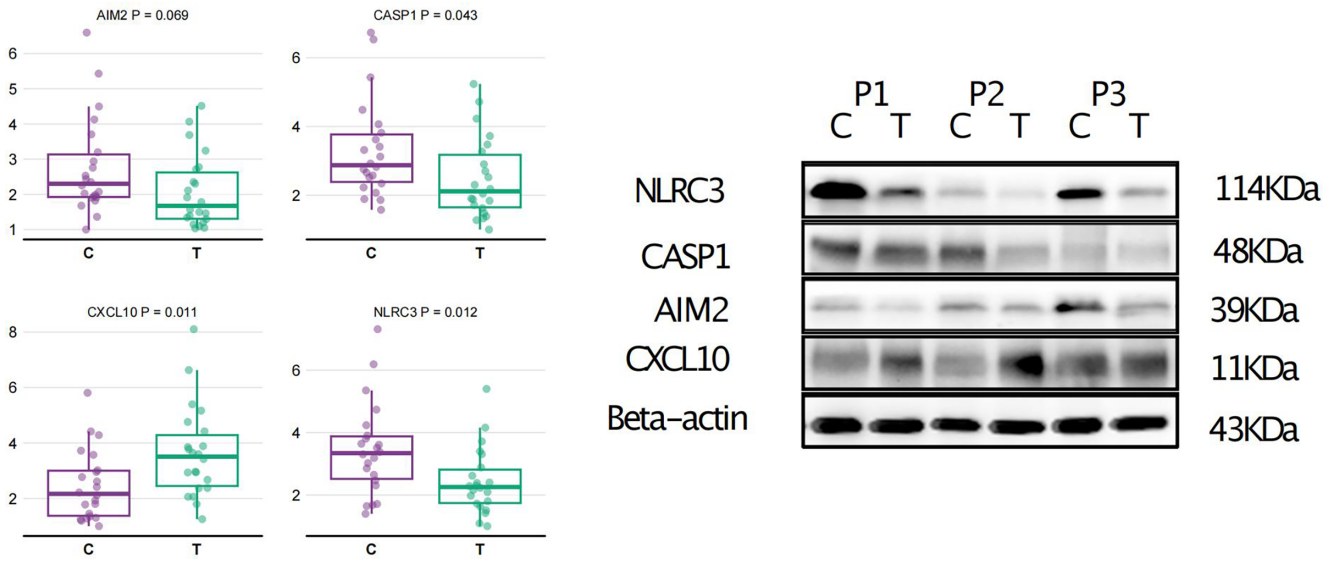

FIGURE 6 Tissue sample validation results of cGAS core genes. (A) Differential expression validation of cGAS core genes using PCR on tissue samples. (B) Tissue sample validation of cGAS core genes using Western blot.

3.7 | cGAS-STING prognostic model construction

To identify genes with an impact on COAD prognosis, we analyzed TCGA and GEO datasets and performed LASSO regression analysis. Our analysis identified a total of 14 genes included in the model (Figure 8A). Further analysis revealed that high model scores were associated with poor prognosis (Figure 8B). We validated the stability of the prognostic model using the GEO dataset and found that patients with high model scores had a worse prognosis (Figure 8C). We divided the prognostic model into two groups based on expression levels (high and low) to analyze its function. Our analysis revealed that the cGAS-STING prognostic model was predominantly associated with tumor recurrence, and patients with high model scores were more likely to experience tumor recurrence (Table 3). Using Decision Curve Analysis (DCA) curves, we evaluated the clinical usefulness of the prognostic model and found that it had good clinical usefulness (Figure 8D). Furthermore, we conducted Cox regression analysis to analyze the impact of conventional clinical parameters and the model grouping on COAD prognosis. Our analysis revealed that the cGAS-STING prognostic model, along with whether radiotherapy was received, significantly impacted the prognosis of COAD patients. Based on these two indicators, we constructed a nomogram for clinical use (Figure 8E).

To further understand the impact of the cGAS-STING prognostic model on COAD, we analyzed the relationship between the model and immune cells (Figure 9A). Our analysis revealed that multiple immune cells, including B cells and T cells, showed differences between the different prognostic groups (Figure 9B). Given that there are many characteristic pathways involved in tumor development, we also analyzed which tumor characteristic pathways were mainly affected by the prognostic model. Using the GSEA database, we obtained 50 tumor characteristic pathways, and after analysis, we found that the cGAS-STING prognostic model was associated with a

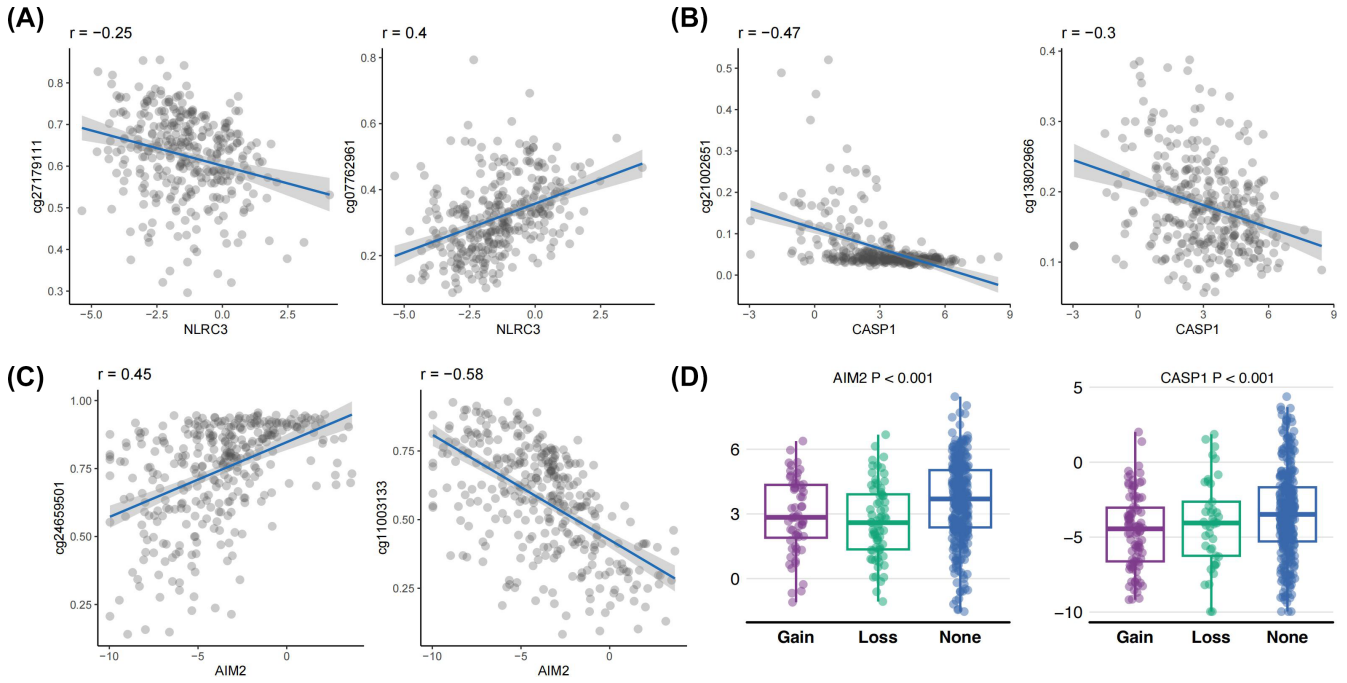

FIGURE 7 Prediction of potential regulatory functions of core genes. (A) Analysis of the correlation between NLRC3 and methylation sites; (B) analysis of the correlation between CASP1 and methylation sites; (C) analysis of the correlation between AIM2 and methylation sites; (D) relationship between CASP1 and AIM2 gene expression and copy number.

total of 36 tumor characteristic pathways, including the toll-like receptor signaling pathway and Wnt signaling pathway (Figure 9C).

3.8 | Impact of NLRC3 overexpression on cancer cell proliferation and apoptosis

In a series of experiments investigating the role of NLRC3 in colorectal cancer, overexpression of NLRC3 was found to promote apoptosis while inhibiting invasion and migration of cancer cells. Scratch assays demonstrated a significant reduction in cell movement in the NLRC3-overexpressing group compared to control groups, indicating that NLRC3 plays a crucial role in suppressing cell motility (Figure 10A). Furthermore, apoptosis assays showed an increase in cell death among the NLRC3-overexpressing cells, underscoring its potential as a pro-apoptotic factor (Figure 10B). Additionally, quantitative PCR analysis revealed a notably higher TP53AIP1 expression level in the NLRC3-overexpressing group compared to the control group, suggesting a regulatory relationship between NLRC3 and TP53AIP1 (Figure 10C). These findings collectively highlight the dual roles of NLRC3 in enhancing apoptosis and restraining the invasive characteristics of colorectal cancer cells.

4 | DISCUSSION

The cGAS-STING pathway is a crucial intracellular immune sensing system that plays a critical role in identifying infection or abnormal cell death inside or outside the cell. When DNA damage or viral infection occurs in the cell, the cGAS protein is activated, leading to the formation of a cGAS-DNA complex that can activate the STING protein.[2–4] This activation triggers a series of immune response processes, including the production of interferon, a crucial molecule in antiviral and anticancer immune responses. Interferon promotes the development and activation of immune cells, enhancing their ability to attack pathogens and cancer cells.[1] Additionally, STING activation can promote cell apoptosis, which is a crucial mechanism for preventing infection and tumor growth while providing space for the growth of new cells. This study focuses on exploring the relationship between cGAS-STING pathway-related genes and COAD across different omic aspects.[5,6,14] Through this exploration, we hope to deepen our understanding of the role of this pathway in COAD and potentially identify novel therapeutic targets.

Firstly, we observed whether cGAS-STING could affect the progression and prognosis of COAD in different omic aspects. Among gene mutations, we found 99 genes with mutations, of which CASP8 and RIPK1 mutations

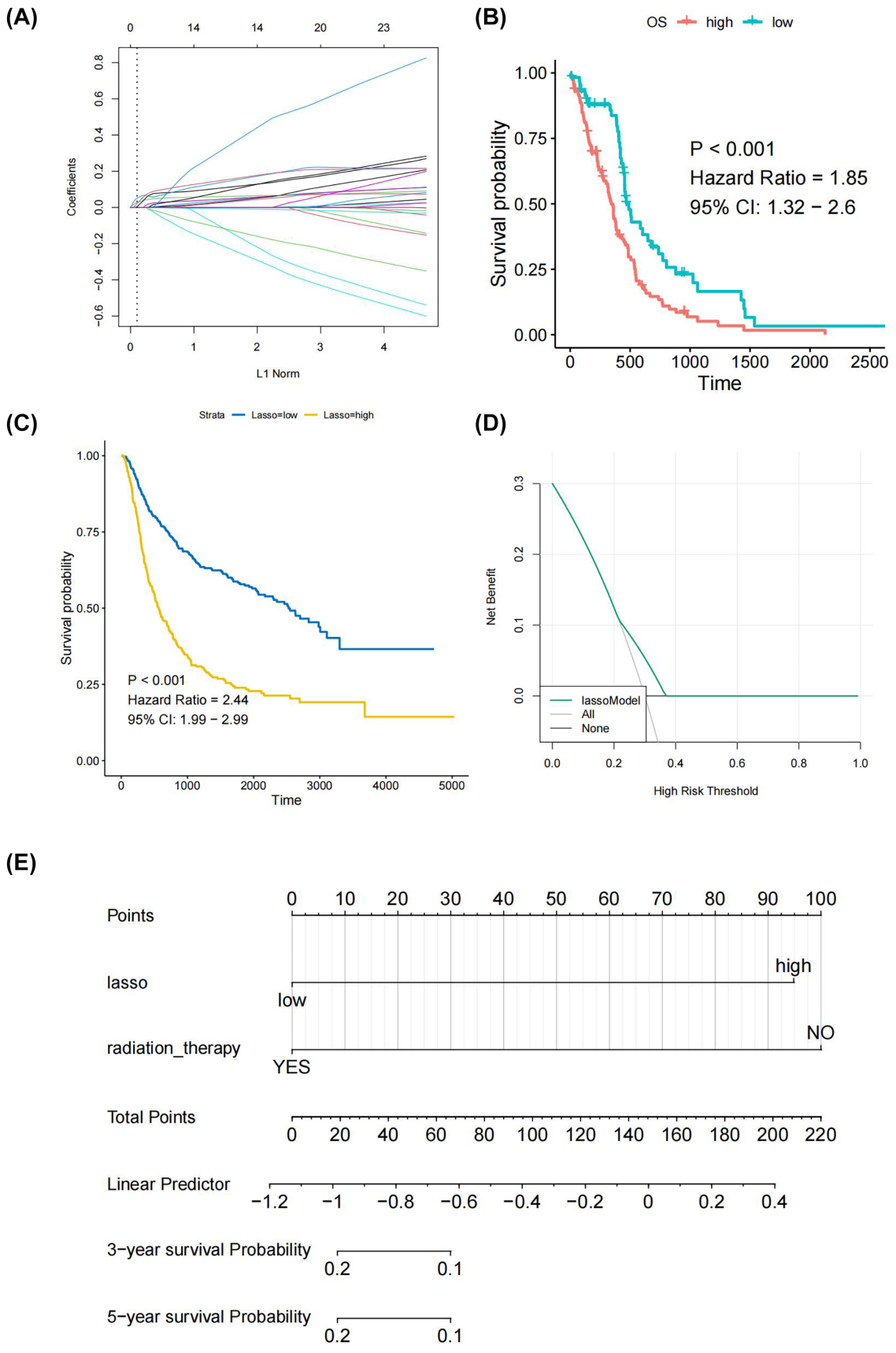

FIGURE 8 Legend on next page.

TABLE 3 Association between cGAS-STING prognostic model scores and tumor recurrence-related biomarkers.

Characteristic	High, $N = 107$[a]	Low, $N = 59$[a]	p-Value[b]
Age			.46
<60	48/107 (45%)	30/59 (51%)	
≥60	59/107 (55%)	29/59 (49%)	
Pharmaceutical_therapy	13/95 (14%)	10/51 (20%)	.35
Unknown	12	8	
Radiation_therapy	17/99 (17%)	4/52 (7.7%)	.11
Unknown	8	7	
Gender			.74
Female	39/107 (36%)	20/59 (34%)	
Male	68/107 (64%)	39/59 (66%)	
Recurrence	17/67 (25%)	2/29 (6.9%)	.037
Unknown	40	30	

[a] n/N (%).
[b] Pearson's Chi-squared test.

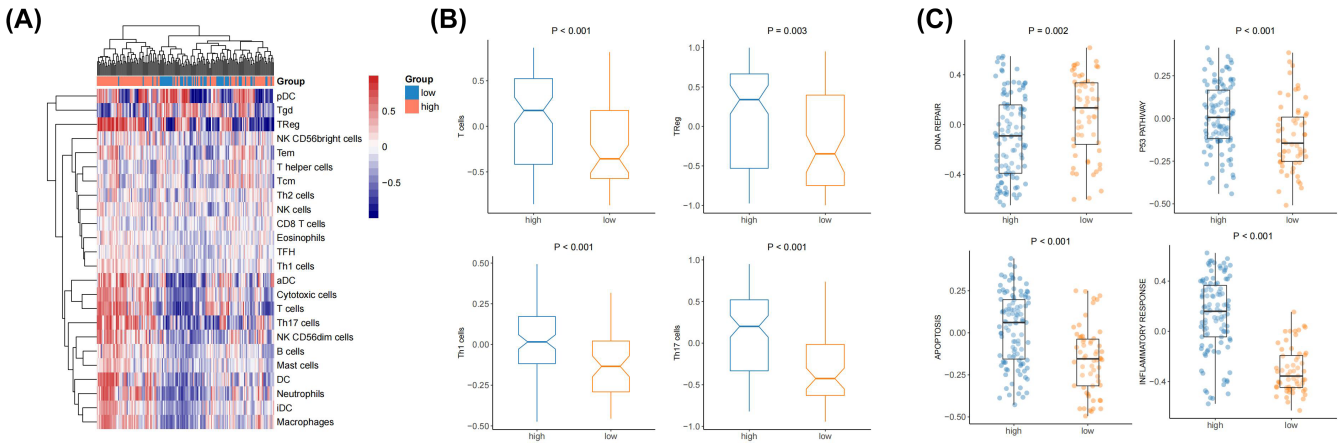

FIGURE 9 The impact of the cGAS-STING prognostic model on immune cell function. (A) Heatmap of differential analysis between the cGAS-STING prognostic model and immune cells. (B) Differences in various types of T cells exist in the cGAS-STING prognostic model. (C) Pathways including apoptosis exhibit differential expression in different prognostic groups.

were associated with the prognosis of COAD. In terms of copy number variations, 99 genes exhibited changes in copy numbers, and mutations in the CASP8 and RIPK1 genes also affected the prognosis of COAD. We found 38 methylation sites with differences between cancer and normal tissue, and the promoter methylation of 37 genes affected the prognosis of COAD. At the mRNA expression level, a total of 38 genes were found to affect the prognosis of COAD. Across all four omic levels, we consistently observed that mutations in the CASP8 and RIPK1 genes had an effect on the prognosis of COAD. CASP8 encodes Caspase 8, an important molecule in the apoptosis signaling pathway. When cells are stimulated externally or internally, Caspase 8 is activated and can lead to cell apoptosis. In addition, Caspase 8 can regulate the immune system and inflammatory response, and participate in various processes of cell growth and differentiation.[15,16] In colorectal cancer, deletion or mutation of

FIGURE 8 Construction of cGAS-STING prognostic model. (A) Construction of cGAS-STING prognostic model; (B) effect of the cGAS prognostic model on the prognosis of TCGA- colorectal adenocarcinoma (COAD) patients; (C) effect of the cGAS prognostic model on the prognosis of new data; (D) DCA curve indicates that the prognostic model has certain clinical usefulness; (E) construction of a nomogram based on the cGAS prognostic model and whether radiotherapy was performed.

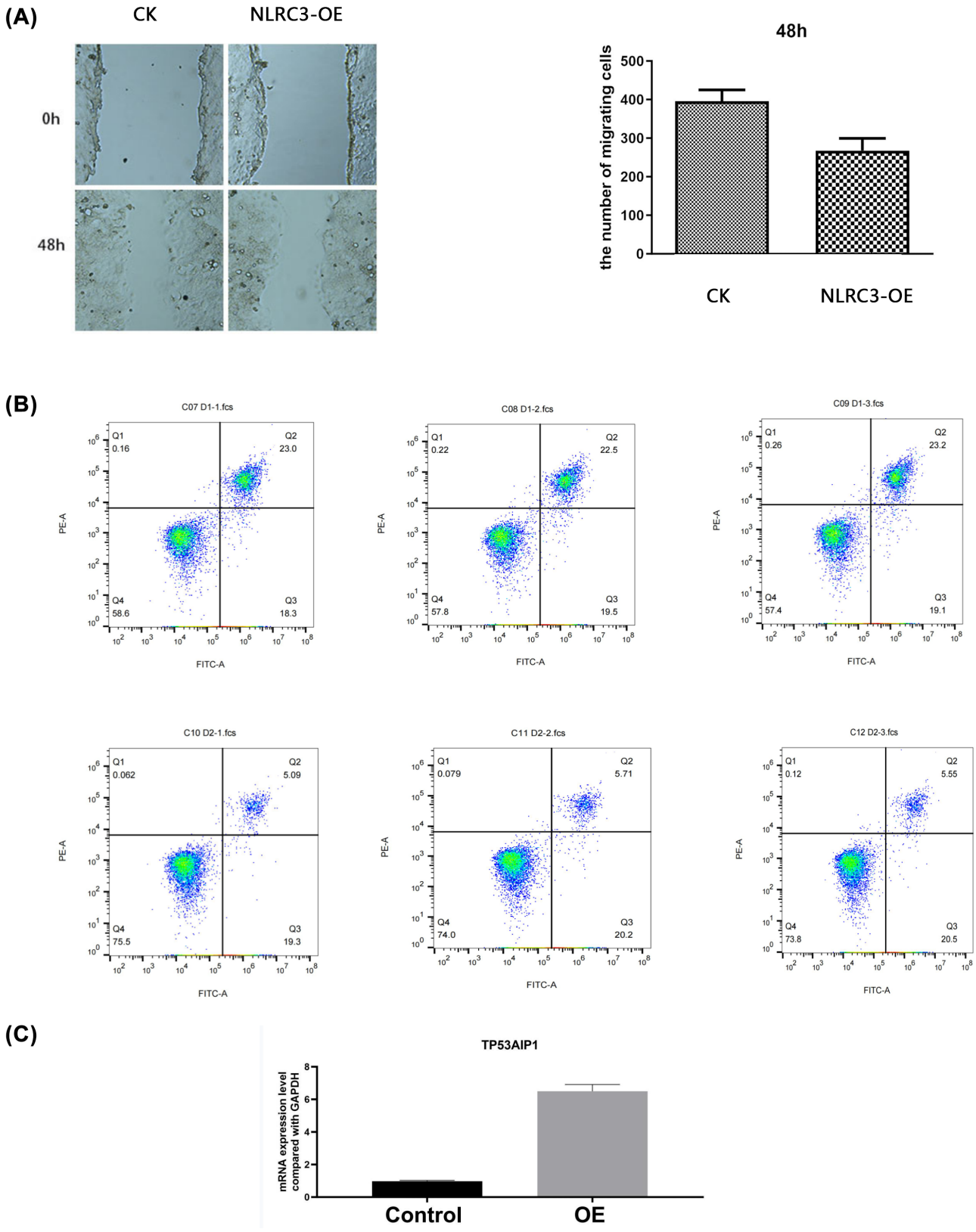

FIGURE 10 NLRC3 affects tumor proliferation and apoptosis. (A) NLRC3 overexpression inhibits cell motility. (B) NLRC3 overexpression is associated with increased apoptosis in tumor cells. (C) The NLRC3-expressing group showed significantly higher levels of TP53AIP1 expression, which promotes cell apoptosis.

the CASP8 gene is often closely related to the biological behaviors of malignant transformation, invasion, and metastasis of cancer cells. Prospective studies have shown that loss or dysfunction of the CASP8 gene is associated with significantly worse prognosis in patients with colorectal cancer.[17] In addition, some studies have found that mutations in the CASP8 gene may affect the results of colonoscopy and reduce the screening effectiveness of colorectal cancer.[18,19]

The protein encoded by the RIPK1 gene is called Receptor Interacting Protein Kinase 1, which plays a crucial role in cell death pathways, including apoptosis, necrosis, and programmed necrosis. In addition, the RIPK1 protein is involved in the regulation of multiple cell signaling pathways, such as the NF-κB signaling pathway, MAPK signaling pathway, and immune response.[20–22] Recent studies have shown that RIPK1 is closely related to the occurrence, growth, and metastasis of colorectal cancer. For example, researchers found that overexpression of RIPK1 may promote the self-renewal of colorectal cancer stem cells, leading to the proliferation and metastasis of colorectal cancer cells.[23,24] In addition, other studies have found that abnormal expression or mutations of RIPK1 may lead to the escape of colorectal cancer cells from the immune surveillance system, thereby promoting the occurrence and progression of colorectal cancer.[25,26]

In our research, we identified four key genes (NLRC3, CASP1, CXCL10, and AIM2) in the cGAS-STING signaling pathway in COAD. These four genes have been studied to some extent in COAD. The protein encoded by the NLRC3 gene belongs to the NOD-like receptor (NLR) family and participates in immune response and inflammation regulation. Like other members of the NLR family, the NLRC3 protein recognizes and binds to microbial DNA or endogenous DNA of host cells through repeating structural domains, thereby activating immune cells' antibacterial and antiviral response mechanisms. In addition, NLRC3 may also down-regulate the immune response in certain cases, slowing down an excessive inflammatory response.[27–29] Researchers have found that overexpression of the NLRC3 gene may inhibit the division and proliferation of intestinal epithelial cells, thereby slowing down the occurrence and development of colon cancer.[30] Additionally, other studies have found a close correlation between the NLRC3 gene's single nucleotide polymorphisms and the occurrence and prognosis of colon cancer.[31]

The protein encoded by the CASP1 gene is caspase-1, an inflammatory cysteine protease that is involved in the regulation of inflammation and apoptosis. In colorectal cancer, studies have shown that its overexpression in tumor cells may lead to overactivation of inflammatory reactions, thereby promoting the proliferation and metastasis of tumor cells.[32–34] The protein encoded by the CXCL10 gene is interferon-induced protein 10, which participates in the process of immune cell aggregation and the release of pro-inflammatory cytokines. In colorectal cancer, studies have shown that the overexpression of CXCL10 in tumor tissue may be closely related to the occurrence and prognosis of cancer.[35–37] The protein encoded by the AIM2 gene is absent in melanoma 2, an inflammasome-related protein involved in the recognition of viruses and bacteria, inflammation regulation, and cell apoptosis. In colorectal cancer, studies have shown that the loss of the AIM2 gene may lead to an excessive colonization of intestinal pathogens, thereby increasing the risk of malignant transformation of intestinal epithelial cells.[38]

To further investigate the impact of the cGAS-STING signaling pathway on COAD prognosis, we constructed a prognostic model based on cGAS-STING genes that affect COAD prognosis. In the cGAS-STING prognostic model, we found that genes with high model scores have a better prognosis. To explore the potential mechanism of cGAS-STING prognosis, we analyzed the relationship between the prognostic model and immune cells. The analysis revealed a correlation between the cGAS-STING prognostic model and various immune cells, including T cells, which are consistent with our previous understanding. The activation of the cGAS-STING pathway can enhance the response of already activated T cells and further induce new T cell responses.[5,6,14] Simultaneously, the activation of the cGAS-STING pathway can enhance Th1 differentiation and promote the production of this specific cell subset through cytokines such as Interleukin 12 (IL-12) and Interferon-γ (IFN-γ), thereby facilitating immune response.[39,40] Furthermore, it has been demonstrated that B cells can also be activated via the cGAS-STING pathway. During the activation process of this pathway, B cells synthesize and secrete more antibodies and enhance their ability to respond to pathogens.[41,42]

In summary, we analyzed the relationship between the cGAS-STING signaling pathway and COAD from multiple perspectives. It provides a new angle and direction for the future study of the cGAS-STING signaling pathway and the mechanism of COAD.

CONFLICT OF INTEREST STATEMENT

All of the authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

All of the data in this article were used in the TCGA datasets (https://www.cancer.gov/about-nci/organization/ccg/reserach/structural-genomics/tcga).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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