RNA m$^5$C regulator-mediated modification patterns and the cross-talk between tumor microenvironment infiltration in gastric cancer

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The effect of immunotherapy strategy has been affirmed in the treatment of various tumors. Nevertheless, the latent role of RNA 5-methylcytosine (m$^5$C) modification in gastric cancer (GC) tumor microenvironment (TME) cell infiltration is still unclear. We systematically explore the m$^5$C modification patterns of 2,122 GC patients from GEO and TCGA databases by 16 m$^5$C regulators and related these patterns to TME characteristics. LASSO Cox regression was employed to construct the m$^5$Cscore based on the expression of regulators and DEGs, which was used to evaluate the prognosis. All the GC patients were divided into three m$^5$C clusters with distinct gene expression characteristics and TME patterns. GSVA, ssGSEA, and TME cell infiltration analysis showed that m$^5$C clusters A, B, and C were classified as immune-desert, immune-inflamed, and immune-excluded phenotype, respectively. The m$^5$Cscore system based on the expression of eight genes could effectively predict the prognosis of individual GC patients, with AUC 0.766. Patients with a lower m$^5$Cscore were characterized by the activation of immunity and experienced significantly longer PFS and OS. Our study demonstrated the non-negligible role of m$^5$C modification in the development of TME complexity and inhomogeneity. Assessing the m$^5$C modification pattern for individual GC patients will help recognize the infiltration characterization and guide more effective immunotherapy treatment.

KEYWORDS
5-methylcytosine (m$^5$C), RNA methylation modification, tumor microenvironment, immune, prognosis, m$^5$Cscore
Introduction

As a global disease, gastric cancer (GC) is the fifth most diagnosed malignancy and the third most common cause of cancer-related death, with 784,000 deaths worldwide in 2018 (1). Although the incidence and mortality rates of GC have declined in several countries, regions seriously threatened by GC, such as China and other East Asian countries, still bear severe health and economic burden. In China, 562,000 newly diagnosed GC patients were recorded, accounting for nearly half of the new cases worldwide (2). The 5-year survival rate of GC is 35.9% in China due to the late stage at diagnosis, notably lower than 71.5% in South Korea and 65% in Japan (3, 4). Due to the complexity of the pathogenic mechanism and the lack of specific biomarkers of GC, the effects of treatment strategies such as surgery, chemotherapy, and radiotherapy are not satisfactory.

Recently, immunotherapy, anti-PD-L1 antibody, and anti-PD-1 antibody have increased the overall survival rate of some advanced GC patients who were treated with two or more lines of chemotherapy (23). The efficiency of immunotherapy depends on the status of EB virus infection, microsatellite instability (MSI)/mismatch repair (MMR), and the expression of PD-L1. However, the dominant population of immunotherapy is still challenging to identify because of the heterogeneity of GC. Hence, to better analyze the heterogeneity and immunophenotype of patients with GC, it is essential to improve long-term survival. Consistently, epigenetic and genetic variations of malignant cells are the only factors participating in the tumor progression, which is a complex multistep process. Notwithstanding, numerous studies have proved that the tumor microenvironment (TME), where tumor cells survive and grow, is crucial in tumorigenesis and development. The composition of TME is rather complicated, including not only the tumor part but also the stromal cells, macrophages, bone marrow-derived cells (BMDCs), distant recruited cells, secreted factors, and neovascularization (24). The detailed types of cells and cytokines in the TME are complex, including cancer–associated fibroblasts (CAFs), myeloid cells, lymphocytes, chemokines, cytokines, and growth factors. Among these cells, tumor–associated macrophages (TAMs), tumor–associated neutrophils (TANs), myeloid-derived suppressor cells (MDSCs), Tie2-expressing monocytes, and dendritic cells together constitute the tumor–associated myeloid cells (TAMCs) (25). The cross-talk between cancer cells and TME components promotes tumor proliferation and angiogenesis, avoids hypoxia, inhibits apoptosis, and mediates immune tolerance. With the gradual deepening of the understanding of the complexity and diversity of TME, increasing data depict its essential role in immune escape and immunotherapy. Moreover, the TME cell infiltration pattern can predict the response to the immune checkpoint blockade (ICB), which is promising in the tumor treatment strategies (26). Accordingly, particular tumor immunophenotypes are supposed to be validated via thoroughly parsing the TME landscape complexity and heterogeneity (27). As GC is characterized by tumor heterogeneity, it is urgent to identify the dominant population of immunotherapy by the landscape TME cell infiltration.

Lately, mC modification is related to the TME-infiltrating immune cells, and the mechanisms are more complicated than expected. In systemic lupus erythematosus (SLE), abnormal mC mRNAs were identified as relevant to critical immune pathways in CD4+ T cells (28). Another study reported that the eraser TET1 is downregulated via NF-xB signaling pathway activation in breast cancer cells (29). Interestingly, Andries and colleagues found that mC-modified mRNA promoted protein expression by the increased ability of the mRNA to elude downstream innate immune signaling and activation of endosomal Toll-like receptor 3 (TLR3) (30). During virus infection, mC RNA methyltransferases, such as NSUN family proteins, were employed to modify viral RNA and change antiviral host responses (31). All these latest findings reveal the fact that mC modification and regulators may have a further effect on the TME, and previous studies focus only on one or two mC regulators due to the limitation of technologies.
In the present study, the genomic and clinical data of 1,983 GC samples were employed to thoroughly estimate the m\(^5\)C modification patterns and the correlation between m\(^5\)C modification and TME features. Three different m\(^5\)C modification patterns and the specific TME cell infiltration peculiarities were identified. Three immunophenotypes, immune-inflamed, immune-excluded, and immune-desert phenotype, were related to the three m\(^5\)C clusters. Subsequently, a scoring system based on the m\(^5\)C modification pattern was established for individual GC patients.

**Materials and methods**

The detailed materials and methods can be found in the supplementary files (32–37).

**Results**

**Blueprint of genetic variation of m\(^5\)C regulators in GC**

In the process of dynamic modification, methyltransferases and demethylases work together to keep the balance of the RNA m\(^5\)C modification with the help of the readers. The ideograph of RNA m\(^5\)C modification is shown in Figure 1A. Firstly, the characteristics of somatic mutations and copy number variations (CNVs) of the 16 m\(^5\)C regulators were summarized in GC. Among all the 433 samples from TCGA, 83 (19.17%) patients experienced mutations of m\(^5\)C regulators. We found that the three demethylases exhibited the highest mutation rates, while the readers (YBX1 and ALYREF) hardly showed any mutations (Figure 1B). Moreover, a significant mutation co-occurrence pattern was identified between NSUN2 and NSUN3 (Figure S1B). For CNV analysis, the most prevalent CNV alternation in the regulators was the amplification in copy number, except for NSUN3, TET2, and NSUN7, which were characterized by a high frequency of CNV deletion (Figure 1C). In Figure 1D, the detailed locations of CNV alteration of each m\(^5\)C regulator are recorded on the chromosomes. Notably, we could thoroughly determine GC patients from normal samples based on the expression of the 16 m\(^5\)C regulators (Figure 1E). To further ascertain the relation between the above genetic alternations and the expression of m\(^5\)C regulators, we explored the expression of regulators in both GC and normal tissues. We found that CNVs might be the main factors leading to the abnormal expression of the m\(^5\)C regulators. Regulators with amplified CNV tended to highly expressed in tumor samples (e.g., DNMT1, ALYREF, and NSUN5), and vice versa (e.g., NSUN7 and NSUN6) (Figures 1C, F). The assessment disclosed the heterogeneity of expression and genetic alteration patterns in m\(^5\)C regulators between GC and normal tissues, hinting that the aberrant expression of m\(^5\)C regulators played an essential role in the tumorigenesis and development of GC.

**m\(^5\)C methylation modification patterns mediated by 16 regulators**

A meta-cohort including five GEO datasets (GSE57303, GSE84437, GSE34942, GSE62254, and GSE15459) with full OS and other clinical data was used to identify the expression pattern of 16 regulators. The prognostic values of 16 m\(^5\)C regulators were analyzed through a univariate Cox regression model (Figure S1C and Figure 2A). We found that the readers ALYREF and YBX1 were favorable prognosis factors for GC patients. The cross-talk between 16 regulators and prognostic significance for patients was revealed in the m\(^5\)C regulator network (Figures 2A, B). We noticed that a significant correlation was shown in both the same and different functional category regulators. Interestingly, the correlation of expression is consistent in regulators from the same functional category. However, we found that the relationship in writers is much complicated, such as DNMT1, which is remarkably negatively correlated with NSUN6 and NSUN7 (Figure 2B). In addition, the expression of the readers ALYREF and YBX1 was almost significantly correlated with other regulators. According to the expression of 16 m\(^5\)C regulators, we further explored the m\(^5\)C modification patterns via the ConsensusClusterPlus package, and identified three different modification patterns by the unsupervised clustering method, including 308 patients in m\(^5\)C cluster A, 334 patients in m\(^5\)C cluster B, and 417 patients in m\(^5\)C cluster C (Figures S2A–D and Table S3). The heatmap of the 16 m\(^5\)C regulators in 1,059 GC patients is depicted in Figure 2C. The expression of 16 regulators in three m\(^5\)C clusters was remarkably different. LogRank analysis showed that the prognosis of patients in m\(^5\)C cluster B was better than the other two clusters (Figure 2D).

**TME cell infiltration characteristics in distinct m\(^5\)C modification patterns**

To better understand the biological characteristics among the distinct m\(^5\)C modification clusters, the GSVA enrichment method was conducted. In Figure 2E, m\(^5\)C cluster A is related to the immune suppression process, while m\(^5\)C cluster B is notably enriched in immune full activation pathways, including cytokine–cytokine receptor interaction, natural killer cell-mediated cytotoxicity, antigen processing and presentation, Toll-like receptor signaling pathway, and chemokine signaling pathway. m\(^5\)C cluster C is enriched in carcinogenic and stromal activation pathways, such as ECM receptor interaction, TGF beta signaling pathway, adhesion and gap junction, mTOR, and
MAPK signaling pathways (Figure 2F). Interestingly, TME immune cell infiltration analysis subsequently showed that m^5^C cluster C was rich in resting and naïve immune cells, such as dendritic cells, CD4 memory T cells, mast cells, B cells, and other innate immune cells, by the CIBERSORT method. On the contrary, m^5^C cluster B is characterized by specific immune cell enrichment (Figure 3A, Figure S2E, and Table S4). The correlation of specific m^5^C regulators and immune cell is shown in Figure S2F. To further reveal the TME features, the single-sample GSEA (ssGSEA) analysis of all the 1,059 cases was conducted. In addition to immune cells, more details about immune functions and pathways can be summarized via the ssGSEA method. As shown in Figure 3B, three distinct immune patterns under three m^5^C clusters are identified (Table S5).
Combined with the survival results above, we were surprised to find that mC cluster A belonged to the immune-desert phenotype, characterized by immunological suppression; mC cluster B was classified as immune-inflamed phenotype, which features immune activation and immune cell infiltration; mC cluster C was labeled as immune-excluded phenotype, characterized by stromal activation and innate immune cell infiltration (Figures 2D and 3A, B). These results demonstrated that the interaction among the writers, erasers, and readers might play fundamental roles in distinct mC modification patterns and TME cell infiltration characteristics of individual GC patients.
m5C methylation modification patterns in the ACRG cohort

We focused on the ACRG cohort, a group of 300 GC participants with complete clinicopathological information, to further reveal the biological behaviors and the features of m5C modification patterns. Like the meta-cohort datasets, the ACRG cohort is divided into three distinct m5C modification clusters as well by the unsupervised clustering method (Figures 3A–D and Figures 3C, D). The heatmap based on the expression of 16 m5C regulators shows that m5C cluster A exhibits a high expression of TET2 and NSUN6 and is downregulated in other regulators; m5C cluster B is characterized by the upregulated readers and five writers including NSUN1–4 and DNMT1; m5C cluster C shows high levels of two erasers and four writers (Figure 3C). We found that patients in m5C cluster A were rich in the diffuse
EMT molecular subtypes were divided into m5C cluster A and B. Analysis showed that all the genes, including CTLA-4, PD-1, and PD-L1, are remarkably highly expressed in m5C cluster A. HLA-A, B, C, E, F, and G, are remarkably highly expressed in m5C cluster B. The survival results revealed that patients in m5C cluster B are related to a favorable prognosis, while m5C clusters A and C show a shorter survival time (Figure 3F). Notably, we also found that the relapse-free survival (RFS) of m5C cluster B is better than the other two clusters (Figure 3G). The findings above demonstrate that most GC patients with EMT molecular subtypes were divided into m5C cluster A and related to stromal activation; most patients with MSI instead of the EMT subtype were in m5C cluster B and characterized by immune activation.

Immunomodulatory effect of m5C modification on the TME

Subsequently, four gene clusters belonging to distinct immune processes were used to reveal the role of m5C modification on the immune regulation of the TME. Chemokines and cytokines with different functions were selected from the published literature. The essential members of human leukocyte antigen (HLA), the major histocompatibility complex (MHC) of human beings, present antigen and mediate immune response. CD8A, CXCL9, CXCL10, GZMA, GZMB, IFNG, PRF1, TBX2, and TNF are related to immune activation. CD80, CD86, HAVCR2, CTLA-4, LAG3, IDO1, PD-1, PD-L1, PD-L2, TNFRSF9, and TIGIT are considered to associate with immune checkpoints. ACTA2, CLDN3, COL4A1, SMAD9, TGRB1, TGFBR2, TWIST1, VIM, and ZEB1 are supposed to correlate with immunomodulatory effect of m5C modification on the TME. Chemokines and cytokines with different functions were selected from the published literature. The essential members of human leukocyte antigen (HLA), the major histocompatibility complex (MHC) of human beings, present antigen and mediate immune response. CD8A, CXCL9, CXCL10, GZMA, GZMB, IFNG, PRF1, TBX2, and TNF are related to immune activation. CD80, CD86, HAVCR2, CTLA-4, LAG3, IDO1, PD-1, PD-L1, PD-L2, TNFRSF9, and TIGIT are considered to associate with immune checkpoints. ACTA2, CLDN3, COL4A1, SMAD9, TGRB1, TGFBR2, TWIST1, VIM, and ZEB1 are supposed to correlate with immune checkpoints. ACTA2, CLDN3, COL4A1, SMAD9, TGRB1, TGFBR2, TWIST1, VIM, and ZEB1 are considered to associate with TGF-β and EMT pathways (24, 38). In Figure 4A, HLA-I molecules, including HLA-A, B, C, E, F, and G, are remarkably highly expressed in m5C cluster B, which means stronger antigen presentation and tumor-killing ability. We noted that HLA-II molecules, such as HLA-DPB2, HLA-DQA1, HLA-DQB2, and HLA-DQA1, were upregulated in m5C cluster A. HLA-G is reported to suppress the immune response and leads to long-term immune escape and tolerance (39). Meanwhile, we also found that the expression of genes related to TGF-β and EMT pathways was remarkably upregulated in m5C cluster A, which added the evidence of stromal activation, while m5C cluster B exhibited overexpression of mRNAs related to immune activation (Figures 4B–D). Immune checkpoint analysis showed that all the genes, including CTLA-4, PD-1, and PD-L1, were upregulated in m5C cluster B (Figure 4C). The results above demonstrate that m5C modification patterns are significantly related to TME immune regulation and may play crucial roles in immunotherapy. However, these findings were only based on the 16 m5C modification regulators.

Considering the heterogeneity and complexity of m5C methylation modification, we tried to identify the DEGs under different m5C clusters using the limma package. Finally, 229 m5C phenotype-related DEGs were found and showed a distinct expression pattern under three m5C clusters (Figures 4E, F). The GO and KEGG enrichment analysis of the 229 DEGs showed that (Figures 5E, F) the DEGs were rich in immune-related biological processes and pathways, including CD8+ θβT cell activation, negative regulation of the immune system process, NOD-like receptor signaling pathway, and TNF signaling pathways.

Generation of m5Csore and capability to predict prognosis

We established a scoring system that depended on the expression of DEGs and m5C regulators to quantify the individual m5C modification pattern; we termed this m5Csore. The univariate Cox regression method was employed to determine the DEGs that were significantly related to the survival of GC patients in ACRG (Table S6). Ninety-nine genes were involved in the LASSO Cox regression algorithm to generate the m5Csore signature, and eight genes were selected, including seven DEGs (RBPMS2, TNFRSF11A, NBEA, INHBB, RGN, DFNAS5, and TPD52L1) and one writer (DNMT3A) (Figures 5A, B). The m5Csore of each GC patient and prognostic information is summarized in Table S7. The alluvial diagram shows the attribute changes of individual GC patients (Figure 5C). Log-rank results depict that the OS of patients with a low m5Csore is remarkably better than patients with a high m5Csore under the cutoff value of 9.92 (Figures 5D, E). The area under the curve (AUC) is 0.766, quantified by the pROC package (Figure 5F). Univariate and multivariate analysis demonstrates that age, N stage, M stage, and m5Csore are the independent factors of prognosis (Figures 5G, H). Meanwhile, we found that m5Cscores significantly differed in distinct ACRG molecular subtypes. Patients in the EMT subgroup showed the highest m5Csore compared to the other molecular groups (Figure 6A). Additionally, patients in m5C cluster B showed the lowest m5Csore compared to other clusters (Figure 6B). In Figure 3G–I, GC patients with a high m5Csore show a significantly higher stromal score and a lower tumor purity score. The results added to the evidence that a low m5Csore was significantly related to immune activation and a high m5Csore was correlated with stromal activation. m5Csore could be a better marker to estimate the m5C modification of individual GC patients. Notably, patients with a low m5Csore and who received adjuvant chemotherapy showed significant treatment advantages (Figure 6C). The result also demonstrated that the prediction value of m5Csore was not affected by chemotherapy, and a low m5Csore showed obvious survival advantage, regardless of whether patients received chemotherapy or not (Figure 6C).
Moreover, older patients, intestinal histological subtype, and early GC patients were notably related to a low m5C score, which demonstrated that these GC patients were characterized by the m5C cluster B and immune-inflamed phenotype, with a better prognosis (Figure 6D).

Validation of m5C modification in TCGA and other datasets

Data from the TCGA-STAD cohort and GEO were used for external and internal validation to determine the role of m5C modification and the prognostic value of m5C score. m5C score was employed to evaluate the individual m5C modification of the single patients in the TCGA dataset, among which 267 patients have a low m5C score and 69 patients have a high m5C score. Combined with the prognosis information, we revealed that patients with a low m5C score and chemotherapy experienced the worst prognosis, while patients with a low m5C score and chemotherapy showed a favorable prognosis (Figure 6G). As shown in Figures 6H, I, patients in the
High-m5Cscore group exhibit less extensive tumor mutation burden than patients in the low-m5Cscore group, with alternation rates of 88.41% and 92.88%, respectively. TMB analysis demonstrated that a high m5Cscore was significantly related to lower TMB, and showed a notable negative correlation (Figures 6H, I). Furthermore, the mean TMB of patients with a high or low m5Cscore was 2.31 and 1.26 mutations per MB. The violin plot also demonstrated that the TMB of patients in the high-m5Cscore group was significantly higher than that of patients in the low-m5Cscore group, and the p-value was 0.012 (Figure S3J).

Next, to further validate the stability of the m5Cscore system, the m5Cscore model was applied to other independent GC cohorts to confirm the prognostic value. Figures 7A–C show that GC patients with a low m5Cscore have a better prognosis in GSE57303, GSE84437, and GSE 15459. Moreover, we combined all the five GEO datasets together and found that the m5Cscore model was validated (Figure 7D). The ROC curve was drawn, and all AUCs were over 0.6 (Figure 7E). In addition, GSE26253, a new GEO dataset, was used to evaluate the predictive value of recurrence-free survival. Figure 7F confirms the ability of
m$^5$Cmethylation to predict RFS, which means the underlying potential mechanisms exist between m$^5$C modification and tumor relapse to be elucidated.

**Discussion**

Growing evidence revealed that aberrant RNA m$^5$C methylation modification played a crucial role in tumorigenesis, progression, and patient prognosis by means of dynamic RNA epigenetic modification. In the current study, we analyzed that m$^5$C regulators in GC explored the correlation between TME and m$^5$C modification, as well as established an m$^5$Cscore system to evaluate the prognosis of GC patients via the data from GEO datasets and the TCGA-STAD cohort. The m$^5$Cscore model was further validated by internal and external datasets. These findings added clues for understanding the m$^5$C modification of individual GC patients.

Sixteen m$^5$Cmethylation regulators were involved in the analysis, including methyltransferases, demethylases, and RNA
binding proteins. Although the exact number of m^5C regulators and detailed mechanisms of m^5C methylation are far from clear, the existing evidence has validated the essential function of m^5C modification on different types of RNA, physiological, and pathological processes (7, 14). Among all the regulators, 13 regulators are significantly aberrantly expressed with 10 genes upregulated and 3 downregulated in GC samples. NSUN7 and DNMT2 are the only low-expression regulators out of the 11 methyltransferases. Sato et al. reported that NSUN7 was upregulated in low-grade glioma with an unknown mechanism (40). However, in GC, we suppose that the low expression of NSUN7 is caused by the loss of CNV frequency. Mei and colleagues found that NSUN2 was overexpressed in GC, which is consistent with our results, and they further validated that NSUN2 promotes GC cell proliferation via repressing p57(Kip2) in an m^5C-dependent manner (41). In correlation analysis, we noticed that the methyltransferases tended to be related to each other, indicating the underlying interaction of mediating the m^5C methylation modification. As for the readers, ALYREF and YBX1 were remarkably overexpressed in GC patients. Research on bladder cancer, breast cancer, HCC, and oral squamous cell carcinoma revealed that ALYREF and YBX1 were upregulated as well (22, 42–44). Intriguingly, high expression of ALYREF and YBX1 are also significantly correlated with the favorable prognosis of GC patients. All three erasers are notably related to the OS of GC patients despite the fact that only TET3 is significantly abnormally expressed in tumor samples. Based on the expression of 16 m^3C methylation regulators, three m^3C modification patterns were distinguished. The three m^3C cluster B belonged to the immune-inflamed phenotype, showing the activation of adaptive immunity; m^3C cluster C was classified as immune-excluded phenotype, characterized by stroma and immunity activation. The GSVA analysis also revealed that m^3C cluster B is enriched in cytokine–cytokine receptor interaction, natural killer cell-mediated cytotoxicity, antigen processing and presentation, Toll-like receptor signaling pathway, and chemokine signaling pathway. These results added to the evidence that the immune-inflamed phenotype, also known as a hot tumor, is characterized by immune cell infiltration and immune-related signal pathway stimulation in TME (45, 46). Additionally, we found that the immune checkpoints in m^3C cluster B were notably overexpressed than the other two m^3C clusters, which indicated that patients in m^3C cluster B might benefit from immunotherapy. In the immune-excluded phenotype, TGF-β and EMT pathways are activated and abate the efficiency of immunotherapy (47). However, we observed the activation of TGF-β and EMT pathways in m^3C cluster A, which was
classified as the immune-desert phenotype. The anomaly may be due to the limited number of TGF-β and EMT pathway-related genes, which requires more data analysis and illustrates the complexity of m^5C methylation modification. In survival analysis, m^5C cluster B showed the most favorable prognosis, which is consistent with the above-mentioned immune-inflamed phenotype.

The m^5Cscore system was established based on the expression of eight genes via the LASSO Cox regression method, namely, DNMT3A, RBPM52, TNFRSF11A, NBEA, INHBB, RGN, DFNA5, and TPD52L1. Among all the genes calculated in the m^5Cscore system, only DNMT3A is an m^5C modification regulator; TNFRSF11A, INHBB, and DFNA5 are involved in TNF-related pathways (48–50); TPD52L1 participates in cell proliferation and calcium signaling; and RBPM52, as an RNA binding protein, is involved in the regulation of cell differentiation and proliferation (51, 52). m^5Cscore is a reliable marker to evaluate the prognosis of GC patients with an AUC of 0.766 in the ACRG training set and 0.898 in the TCGA validation set. m^5Cscore was verified by other GEO datasets as well. Inevitably, m^5Cscore is distinct in different m^5C clusters, in which m^5C cluster B had the lowest m^5Cscore. We noticed that GC patients with the EMT molecular subtype show the highest m^5Cscore, demonstrating poor prognosis. Furthermore, GC patients with a high m^5Cscore tend to have a shorter RFS, indicating that m^5C methylation may play an essential role in tumor recurrence.

**Conclusion**

In summary, we revealed the potential regulatory mechanisms of m^5C methylation modification on the GC TME. The characteristics of distinct m^5C modification patterns might lead to the complexity and heterogeneity of individual GC TME. A far-reaching understanding of specific m^5C modification patterns in GC will contribute to identifying TME cell infiltration and guiding clinical immunotherapy treatments.

**Data availability statement**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**Ethics statement**

The study complied with the principles set forth in the Declaration of Helsinki. Access to the deidentified linked dataset was obtained from the TCGA and GEO databases in accordance with the database policy. For analyses of deidentified data from the TCGA and GEO databases, institutional review board approval and informed consent were not required.

**Author contributions**

All authors searched the literature, designed the study, interpreted the findings, and revised the manuscript. QZ, JS, XS and KS carried out data management and statistical analysis and drafted the manuscript. JL, XG, KS and XQ helped with cohort identification and data management. QZ, JS, XS and KS performed project administration. All authors contributed to the article and approved the submitted version.

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**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**Supplementary material**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.905057/full#supplementary-material
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