Research article

A potential role for metastasis-associated in colon cancer 1 (*MACC1*) as a pan-cancer prognostic and immunological biomarker

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Abstract: *Background:* Metastasis-Associated in Colon Cancer 1(*MACC1*) is a validated biomarker for metastasis and is linked to survival. Although extensive experimental evidence indicates an association between *MACC1* and diverse cancers, no pan-cancer analyses have yet been performed for this marker, and the role of *MACC1* in immunology remains unknown. *Material and Methods:* In our study, we performed the analysis of *MACC1* expression and its influence on prognosis using multiple databases, including TIMER2, GEPIA2, Kaplan-Meier plotter. *MACC1* promoter methylation levels were evaluated using the UALCAN database. Based on the TCGA database, we explored the relationship between *MACC1* and tumor mutational burden (TMB), microsatellite instability (MSI), immune checkpoints using the R programming language. We evaluated the association between *MACC1* and immune infiltration via TIMER and UALCAN. *Results:* Our results revealed that abnormal DNA methylation may be an important cause for the different expression of *MACC1* across cancer types. Meanwhile, we explored the potential oncogenic roles of *MACC1* and found significant prognostic value. *MACC1* may be related to T-cell function and the polarization of tumor-associated macrophages, especially in STAD and LGG. Its expression was associated with immune infiltration and was found to be closely related to immune checkpoint-associated genes, especially CD274 and SIGLEC15, indicating that *MACC1* may be a potential immune therapeutic target for several malignancies. Our paper reveals for the first time the relationship between *MACC1* and cancer immunology. *Conclusions: MACC1* might act as a predictor for the immune response in cancer patients, and could also represent a new potential immunotherapeutic target.

1. Introduction

Cancer is a major cause of death globally; incidence and mortality for this group of diseases are expected to increase in the coming years. A single cell with malignant characteristics acquired through epigenetic alterations can lead to carcinogenesis, and accumulated genetic mutations contribute to cancer progression [1,2]. Mutations at certain genomic locations result in the loss of gene function [3,4]. The significance of individual alterations in gene expression across diverse tumor types is still unclear. Moreover, the survival of patients with primary tumors is directly related to the occurrence of metastasis. Early and accurate identification of those at high risk of metastasis is vital for effective disease management, however, there are currently no clear molecular markers for this group. The identification of novel, reliable biomarkers with significant predictive value is therefore a priority.

The first identification of Metastasis Associated with Colon Cancer 1 (*MACC1*) has drawn great attention through analysis of genome-wide expression in all colorectal cancer patients [5]. Accumulated evidence has proved that *MACC1* has a comprehensive function across many different solid cancer types and across many physiological and pathological processes including cell proliferation, migration, tumorigenesis and metastasis.

In recent years, the link between *MACC1* expression and disease prognosis has been studied in diverse tumor types beyond colorectal cancer [5], such as hepatobiliary cancer [6], pancreatic cancer [7], lung cancer [8], ovarian cancer [9], breast cancer [10], glioblastoma [11]. In a pioneering study, we found that people with cancer who had a high *MACC1* expression also had a survival rate of 15%, much lower than the 80% survival rate of those who had low *MACC1* expression [5]. The laboratory evidence suggests that *MACC1* overexpression accelerates the procession of benign adenoma into malignant tumors and increase hepatic metastases in xenograft models [12,13]. Previous meta-analysis results also confirmed that in multiple solid tumors, *MACC1* expression was strongly related to prognosis, especially in digestive system neoplasms [14–16]. These data provide favorable evidence for *MACC1* as a useful prognostic and predictive marker.

High-throughput molecular data for over 10,000 tumors is publicly available in online database, and many statistical methods for analysis have been established and validated in previous studies. Bioinformatics analyses are a cost-effective and relatively simple way to investigate gene function and provide new insight into potential biological mechanisms underlying tumor development and progression. In this study, we performed a comprehensive bioinformatic analysis of the role of *MACC1* expression across 33 cancer types in the cancer genome atlas (TCGA) database. This is the first pan-cancer analysis to access multiple different aspects of gene expression and function, including DNA methylation, immune infiltration, genetic alteration, and relevant pathways. The insights gained from this study pave the way for subsequent, more specific investigations, and provide a novel overview into the underlying mechanisms of *MACC1* involvement in tumor initiation and progression, and immune infiltrations.

2.   Materials and methods

2.1. Gene expression analysis

The mRNA expression of *MACC1* in normal tissue and protein expression in some major tissues (displayed by immunohistochemistry) were obtained from the Human Protein Atlas (HPA) databases (http://www.proteinatlas.org/) [17]. The antibodies used are HPA020103.

To explore the different *MACC1* mRNA expressions between tumors and normal tissues, we used the "Gene-DE" module of TIMER2 (tumor immune estimation resource, Version 2) web (http://timer.cistrome.org/). TIMER2 is a web resource for analyzing immune infiltrates, and "Gene-DE" is a module for exploring the expression of differential genes in tumors compared to adjacent normal tissues [18].

2.2. Promoter methylation analysis

We used the UALCAN database (http://ualcan.path.uab.edu/index.html database) to explore the *MACC1* promoter methylation profile [19]. The UALCAN portal is a user-friendly web resource for analyzing cancer Omics data. $P < 0.05$ is considered a significant threshold.

2.3. Clinical pathological analysis

The UALCAN database was also used to assess the relationship between *MACC1* mRNA transcription level and pathological cancer stage, which was divided into 4 Stages (Stage 1, 2, 3, 4) [19]. $P < 0.05$ is considered a significant threshold.

2.4. Survival analysis

The prognostic value of *MACC1* mRNA expression in 33 cancers was visualized with two tools separately: GEPIA2(http://gepia2.cancer-pku.cn/), Kaplan-Meier   plotter   (http://kmplot.com/) databases [20]. This analysis was visualized using Kaplan-Meier cubes. GEPIA2 includes RNA sequencing expression data from 9736 tumors and 8587 normal samples from the TCGA and GTEx projects. The cutoff-high (50%) and cutoff-low (50%) values were used as expression thresholds to separate the high expression and low expression queues. We also used the Kaplan-Meier plotter database to evaluate these indicators, including overall survival (OS) and disease-free survival *(DFS)* in certain tumors. $P < 0.05$ was considered statistically significant.

2.5. Tumor mutational burden,   Microsatellite   instability,   Immune checkpoint-associated genes analysis

The mRNA expression data of *MACC1* mRNA expression data across 33 tumor types and normal tissues were downloaded from this TCGA database (https://portal.gdc.cancer.gov/). We computed TMB and MSI scores and analyzed the relationship between them and *MACC1* using R programming language. TCGA is a comprehensive and web-based database that, as of 2018, has generated massive NGS data for more than 11,000 tumors across 33 cancer types. TMB can

measure the number of mutations of a tumor genome and can predict the response to immunotherapy. MSI is a high mutation phenotype caused by frequent polymorphisms in short repeating DNA sequences and single nucleotide substitutions due to DNA mismatch repair (MMR) defects. MSI occurs frequently across tumors and can act as a predictor of sensitivity for immune-therapy [21,22]. In this analysis, the version of R language software was R-4.0.3. P-values less than 0.05 were considered statistically significant.

2.6. Immune checkpoint-associated genes analysis

We extracted the expression values of 8 immune checkpoint-related genes, including SIGLEC15, IDO1, CD274, HAVCR2, PDCD1, CTLA4, LAG3 and PDCD1LG2, and observed the relationship between *MACC1* mRNA expression and these genes. The results were presented as a heatmap. R-4.0.3 software was used for statistical analysis. $P < 0.05$ is taken as the significance threshold.

2.7. Immune infiltration analysis

TIMER is a comprehensive online resource for analyzing the immune infiltrates across all TCGA cancers (https://cistrome.shinyapps.io/timer/) [23]. It is made up of seven modules, including gene, SCAN, mutation, survival, correlation, differential gene expression. First, we input *MACC1* in the "Gene" module of TIMER and evaluated the correlation between infiltration levels of 6 immune cells and *MACC1* expression, which involved B cells, CD4$^+$ T cells, CD8$^+$ T cells, Neutrophils, Macrophages, and Dendritic cells. We showed the expression level of *MACC1* on X-axis and immune cell infiltration level on the Y-axis to plot the scatterplots. In the TIMER database, the expression level of *MACC1* was adjusted to log2(TPM).

Secondly, the immunedeconv, an R package integrating six types of algorithms, was used to estimate the relationship of different infiltrating immune cell types and the *MACC1* expression level of each tumor sample by R package. The results are presented as a heat map. $P < 0.05$ is considered statistically significant. The version of R software using in this article is R-4.0.3.

2.8. Immune cell type markers analysis

The relationship of *MACC1* mRNA expression with immune cell-related markers of B-cells, CD8$^+$ T cells, neutrophils, macrophages, dendritic cells NK cells, Th1 cells, Treg cells, and monocytes in tumors was estimated using the "correlation" module of the TIMER algorithm. Then we verified the correlation of *MACC1* with immune cell markers in LGG and STAD and the P-values and partial correlation (Cor) values were obtained after tumor purity and the patient's age-adjusted Spearman's rank correlation test.

2.9. Gene mutation analysis

The cBio Cancer Genomics Portal (http://cbioportal.org) was used to analyze the frequency alteration of *MACC1*, including mutation, amplification, deep deletion, and multiple alterations. The cBioPortal database is an online resource to explore the multidimensional cancer genomics data [24].

2.10. MACC1 copy number variations and immune infiltrates analysis

We searched *MACC1* in the SCAN module of TIMER, which includes deep deletions, arm-level deletions, normal alterations, arm-level gains, and high amplification [23]. In this module, we confirmed the association of the mRNA expression level of *MACC1* and immune infiltration level with different somatic copy number alterations. A bilateral Wilcoxon rank-sum test was used to compare the infiltration levels of each SCNA category with normal. Box plots are used to display the distributions of each immune subpopulation of immune cells at different copy numbers. The significance threshold is set as P-value < 0.05.

3. Results

3.1. Gene expression analysis

MACC1 is located on the P-arm complementary chain of human chromosome 7 (7p21.1) (Figure S1A). The ZU5-DD architecture was observed in *MACC1* and conserved among different species (e.g., Homo sapiens, Rattus norvegicus, etc.) The presence of carbon terminal death domains (DD) in *MACC1*, indicates that this gene is involved in the regulation of cell apoptosis (Figure S1B). We also use the phylogenetic tree date to demonstrate the evolutionary relationship of *MACC1* among species (Figure S2).

First, we explored the mRNA expression profile of *MACC1* in normal tissues. As shown in Figure 1A, based on a combination of HPA (human protein map), GTEx, and FANTOM5 (functional annotation of mammalian genome 5) data sets, *MACC1* is highly expressed in the digestive system (such as the esophagus, tongue, colon), especially in the small intestine, relatively low expression in most other organs. The urinary system and reproductive system, including the kidney and uterus, also showed relatively high expression levels.

We accessed mRNA level as a proxy for *MACC1* expression in various cancer types using data from TCGA database. *MACC1* expression was observed in all tumor types, but the extent of expression was found to vary (Figure 2A). *MACC1* expression in the tumor tissues of CHOL (cholangiocarcinoma), COAD (Colon adenocarcinoma), GBM (glioblastoma multiforme), KIRP (Kidney renal papillary cell carcinoma), LUAD (lung adenocarcinoma) (P < 0.001), ESCA (esophageal carcinoma) (P < 0.01), CESC (cervical and endocervical cancer), BLCA (bladder urothelial carcinoma) (P < 0.05) was much higher than the normal tissues. Additionally, compared to the adjacent normal tissues, *MACC1* expression was significantly lower in HNSC (Head and neck squamous cell carcinoma), KICH (Kidney chromophobe), KIRC (Kidney renal clear cell carcinoma), LIHC (Liver hepatocellular carcinoma), LUSC (Lung squamous cell carcinoma), PARD (Prostate adenocarcinoma (P < 0.001), PCPG (Prostate adenocarcinoma) (P < 0.01). Furthermore, we selected some of the classic immunohistochemical images from HPA databases. These images demonstrated ubiquitous cytoplasmic staining for *MACC1* protein, with low levels of staining in normal tissue and high levels of staining in cancer tissue, including in colorectal cancer and lung adenocarcinoma. Conversely, in prostate cancer, levels of *MACC1* staining were lower than in corresponding normal prostate tissue (Figure 1B).
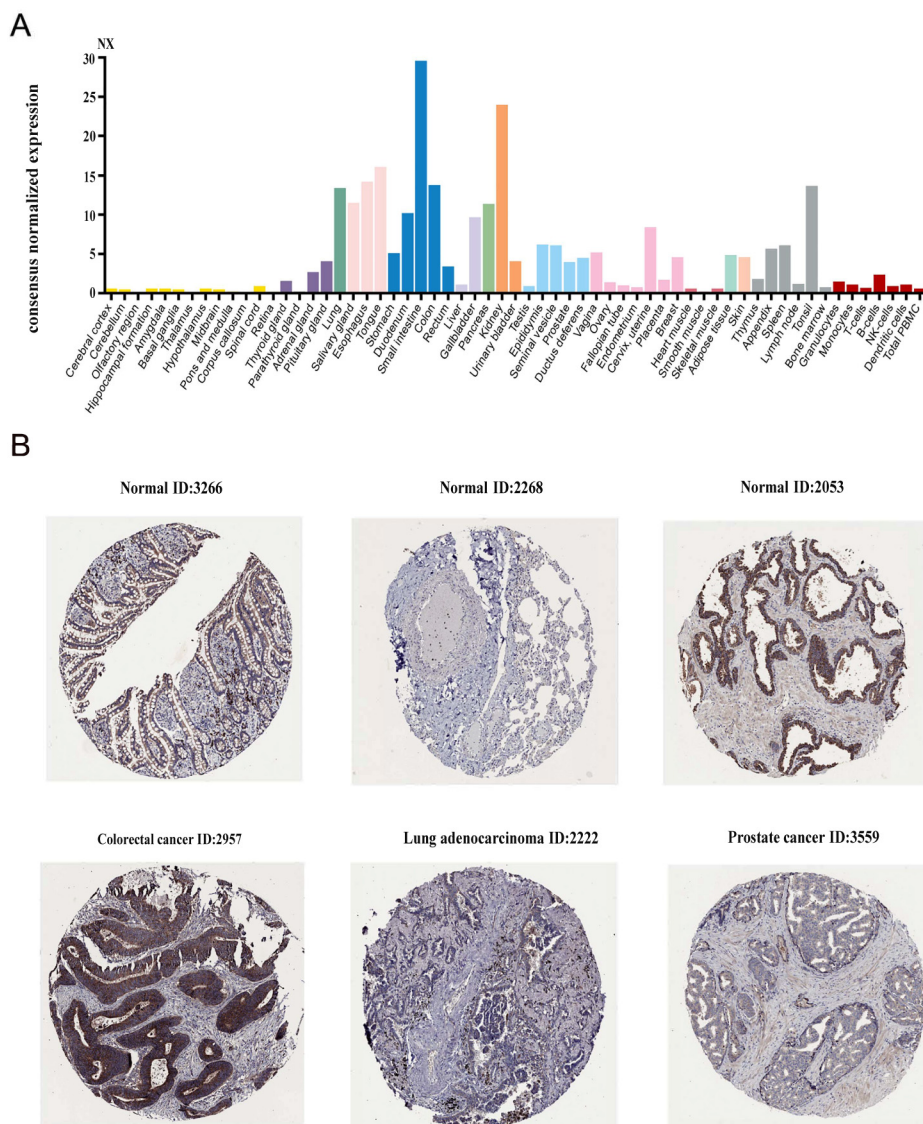
Figure 1. Expression of *MACC1* in HPA database. (A) mRNA expression of *MACC1* in human normal tissues. (B) Immunohistochemistry images of *MACC1* protein in normal tissues and colorectal cancer, lung cancer, prostate cancer in HPA database.

3.2. Correlation between MACC1 DNA methylation and gene expression

To better understand the relevance and underlying mechanisms of *MACC1*, we accessed the correlation between the expression level of *MACC1* and methylation levels of *MACC1* promoter in diverse cancers using ALCAN database (Figure 2B). Combined with the TCGA database, we selected several cancers which showed higher *MACC1* expression compared to normal tissue, including BRCA, BLCA, LUAD, COAD, CHOL, CESE, LUSC, READ, TGCT, THCA, UCEC. And *MACC1* promoter methylation levels were significantly lower in these cancer types than in corresponding normal tissue.
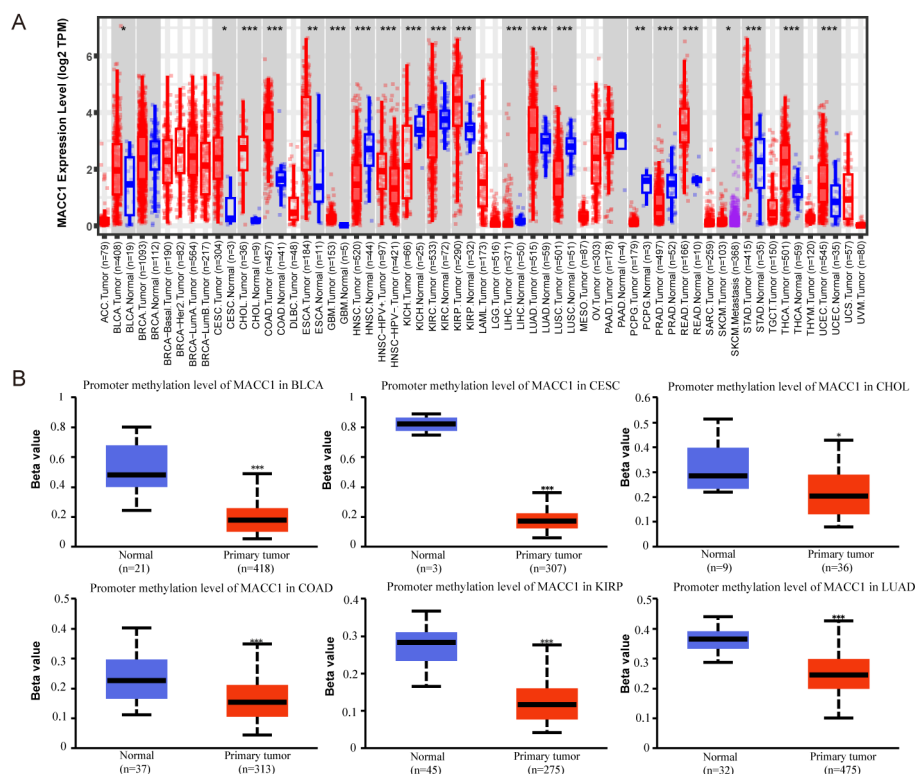
Figure 2. Expression of *MACC1* and methylation level of *MACC1* promoter. (A) The mRNA expression pattern of *MACC1* across diverse tumors, performed using TIMER2. Red represents tumor tissue. Blue represents normal tissue. (B) The methylation level of *MACC1* promoter in six tumor types, visualized using UALCAN database. The y-axis refers to beta value, ranging from 0 (unmethylated) to 1 (fully methylated). Beta value: 0.7~0.5 for hypermethylation, Beta value: 0.3~0.25 for hypomethylation. The first layer asterisk above the error line indicates statistical significance of the difference on comparison with normal tissue group. *P < 0.05, **P < 0.01, ***P < 0.001.

3.3. Identification of potential therapeutic agents targeting MACC1

We analyzed the associated gene-drug interaction network using the comparative toxigenomics database (CTD) and visualized the results using Cytoscape. As shown in Figure S3, various drugs are predicted to affect the expression of *MACC1*. Interestingly, we found that histone deacetylase (HDAC) inhibitors, including Entinostat, Belinostat, and Panobinostat, may increase expression of *MACC1*. This finding provides novel avenues for therapeutic research.

3.4. Association of MACC1 expression in tumors with clinical phenotypes

Further correlation analyses of *MACC1* mRNA expression level with pathological stage were performed for each tumor separately using the UALCAN database. The results showed that *MACC1* expression was significantly different between pathological stages of 15 tumors compared to the normal tissues (Figures 3 and S4). First, we found that different pathological stages of 5 tumors showed lower *MACC1* expression compared with normal tissues, including KICH, KIRC, KIRP, HNSC, LUSC.

Stage IV samples of KICH, KIRC, KIRP were associated with significantly lower *MACC1* expression than earlier stage, indicating that decreased *MACC1* expression may indicate the disease has progressed in these patients. In contrast, in some tumors, its expression was found to be higher in patients with advanced cancer than in patients with earlier stage such as in CESC, ESCA, COAD, UCEC, THCA (Figure 3). In our study, *MACC1* expression was found to be slightly increased in both Stages 3 and 4 versus Stage 2 ($P < 0.001$; $P < 0.05$). The result demonstrated that *MACC1* expressed at higher levels in colon cancer of patients with metastasized compared to the tumor patients in the early stage.
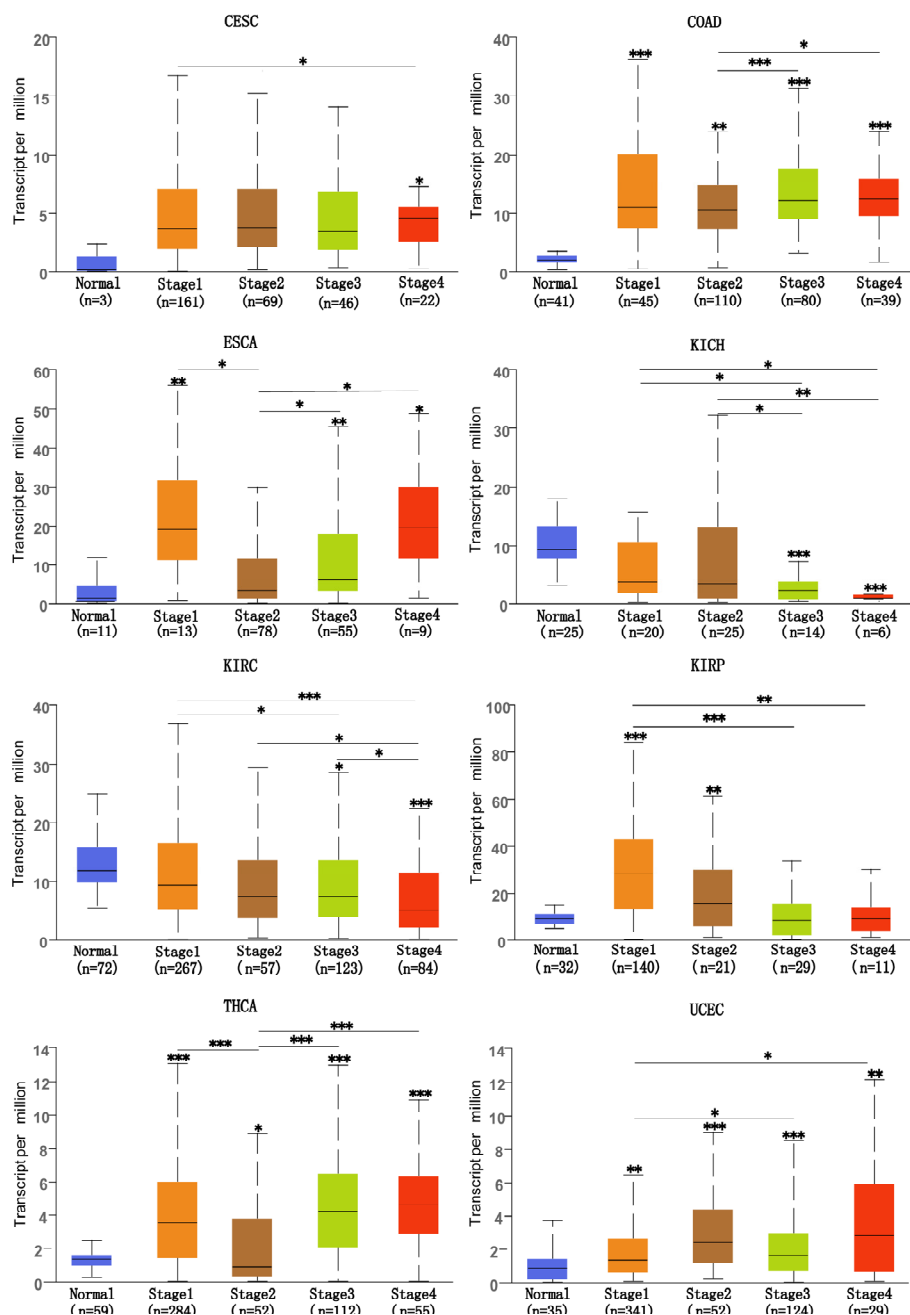

Figure 3. Correlation between MACC1 expression and pathological.

The analysis of relationship between *MACC1* expression and pathological stages was shown in 8 cancer types using UALCAN database, cervical and endocervical cancer (CESC), colon adenocarcinoma (COAD), thyroid carcinoma (THCA), uterine corpus endometrial carcinoma (UCEC), esophageal carcinoma (ESCA), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP). The first layer asterisk above the error line indicates the comparison with normal group, and the layers layer asterisk above the remaining error line represents the comparison between the corresponding groups covered by the line. *P < 0.05, **P < 0.01, ***P < 0.001.

3.5. Association of MACC1 expression in several tumor types with the prognosis

We analyzed the relationship between *MACC1* expression status and the prognosis of patients across cancers. As shown in Figure 4A, stronger expression of *MACC1* was related to poorer prognosis of overall survival (OS) in three cancer types: COAD (log-rank P = 0.0025), LGG (log-rank P = 6.9e-11), UVM (log-rank P = 0.019). In terms of DFS, the results revealed a correlation between high *MACC1* expression and poor prognosis in COAD (log-rank P = 0.019), LGG (log-rank P = 2.6e-05), PAAD (log-rank P = 0.038). Notably, *MACC1* was associated with greater OS in KIRC (log-rank P = 4.1e-07), KIRP (log-rank P = 0.008), PCPG (log-rank P = 0.018), SKCM (log-rank P = 0.0049) and greater DFS in KICH (log-rank P = 0.046), KIRC (log-rank P = 0.00023), KIRP (log-rank P = 0.0031).

Moreover, we investigated the correlation between *MACC1* expression and prognosis across different cancer types in the Kaplan-Meier plotter database based on Affymetrix microarrays. We discovered that high *MACC1* expression may be a predictor of poor prognosis of DFS (P = 5.8e-05) and OS (P = 0.0052) in ovarian cancer (Figure 4B). On the contrary, poorer prognosis in lung cancer and gastric cancer was significantly related to lower expression of *MACC1* (OS: P = 2.9e-07; P = 0.0029, respectively). However, *MACC1* expression had little impact on breast cancer according to the database.
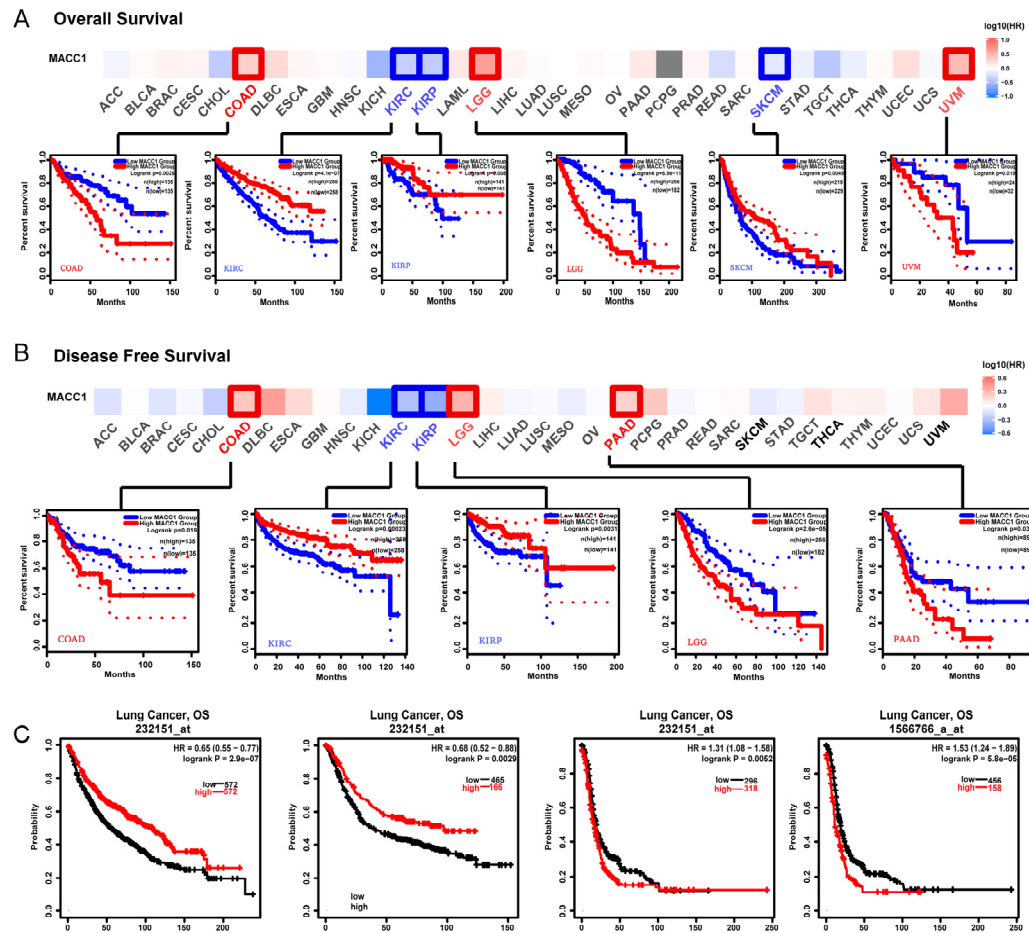
Figure 4. Correlation between *MACC1* expression and survival prognosis of cancers in TCGA. (A) Overall survival analysis. (B) Disease free survival analysis. The survival curve was plotted using GEPIA2 tool, comparing the patient with high and low expression in solid tumors. (C) The positive results of survival curve are displayed using Kaplan-Meier plotter database.

3.6. Association between MACC1 expression, tumor mutational burden, and microsatellite instability.

Table 1 revealed the correlation of *MACC1* and TMB, MSI, which can predict the immunotherapy efficacy. As shown in Figure 5A, we found TMB was negatively related to *MACC1* expression in BRCA, KICH, LIHC, LUAD, PRAD, UCS, UCEC, and COAD (P < 0.0.05), but positively in KIRP, ESCA, HNSC, LGG, and PAAD (P < 0.05). Additionally, MSI was also found to be negatively associated with *MACC1* expression levels in UCEC (P < 0.001) and UCS (P < 0.05), but positively associated with expression of this gene in OV, READ, STAD, TGCT (P < 0.01) (Figure 5B).

Table 1. Correlation analysis of *MACC1* expression and TMB/MSI.

Cancer	TMB		MSI	
	Correlation	*P*-value	Correlation	*P*-value
ACC	-0.142	0.21873	0.071	0.53964
BLCA	0.027	0.59282	-0.003	0.94940
BRCA	-0.073	0.02169	-0.009	0.76597
CESC	0.046	0.57506	-0.026	0.65448
CHOL	-0.199	0.24307	0.242	0.15427
COAD	-0.293	2.48E-08	0.077	0.11088
DLBC	-0.295	0.07668	-0.28	0.05424
ESCA	0.367	3.82E-06	0.139	0.07896
GBM	0.058	0.48026	0.037	0.65588
HNSC	0.153	0.00070	-0.015	0.73890
KICH	-0.251	0.04581	0.14	0.26538
KIRC	-0.033	0.53559	0.079	0.14706
KIRP	0.128	0.03413	0.021	0.72721
LAML	-0.161	0.09980	-0.051	0.59316
LGG	0.343	9.75E-15	-0.072	0.11118
LIHC	-0.141	0.01563	0.001	0.97814
LUAD	-0.173	9.99E-05	0.03	0.49973
LUSC	0.007	0.88178	-0.039	0.38478
MESO	-0.146	0.19437	0.01	0.93062
OV	-0.1	0.11796	0.142	0.01827
PAAD	0.348	7.28E-06	-0.046	0.54665
PCPG	0.043	0.57813	-0.1	0.18305
PRAD	-0.096	0.03472	-0.023	0.60271
READ	-0.176	0.05380	0.195	0.01670
SARC	0.064	0.34035	-0.004	0.94713
SKCM	0.032	0.48829	0.035	0.45072
STAD	0.101	0.05260	0.136	0.00869
TGCT	0.038	0.66762	0.28	0.00106

Cancer	TMB		MSI	
	Correlation	*P*-value	Correlation	*P*-value
THCA	-0.019	0.68334	-0.016	0.71447
THYM	0.092	0.32419	0.012	0.89740
UCEC	-0.253	7.33E-09	-0.2	3.04E-06
UCS	-0.272	0.04268	-0.294	0.02812
UVM	-0.181	0.13749	0.06	0.62210

3.7. Correlation between MACC1 expression and immune checkpoint-associated genes

To find potential patients who can benefit from this treatment, we selected several potential immune checkpoints to explore whether there is a relationship between the immune escape and *MACC1* expression, including SIGLEC15，TIGIT，CD274，HAVCR2，PDCD1，CTLA4，LAG3, and PDCD1LG2. As shown in Figure 5C, expression of all immune checkpoint-associated genes was related to *MACC1* expression in LGG, PRAD, SKCM, UVM. Moreover, most of the genes found closely with *MACC1* expression except for one gene in GBM, LIHC, SARC, TGCT. Most cancers showed a significant correlation with the immune checkpoint. Our analyses in STAD revealed that *MACC1* expression is correlated with that of HAVCR2, LAG3, SIGLEC15, TIGIT. Interestingly, SIGLEC15 has a strong association with *MACC1* expression in 21 types of cancer.
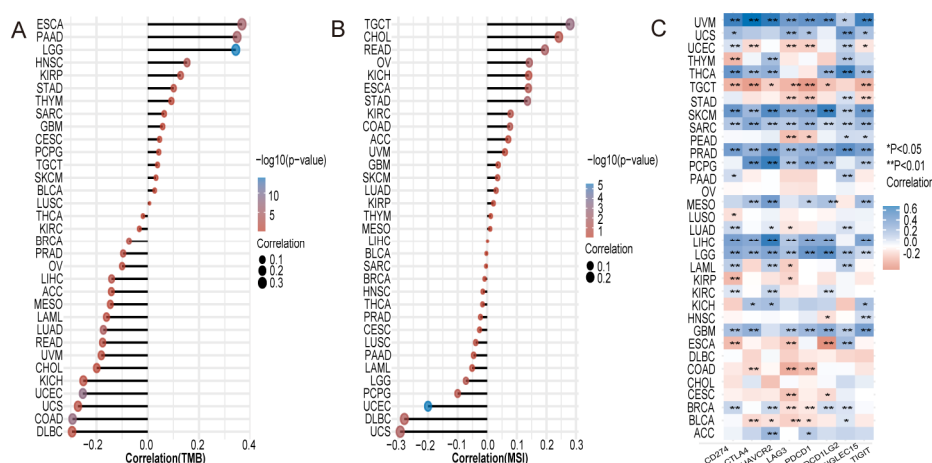
Figure 5. Correlation between *MACC1* expression and tumor mutational burden, microsatellite instability, immune checkpoint-associated genes. (A) Based on the TCGA database, the tumor mutational burden was explored using R language. (B) Based on the TCGA database, the microsatellite instability was explored using R language. (C) Correlation analysis of *MACC1* expression and immune checkpoint-associated genes. *P < 0.05, **P <0.01, ***P < 0.001.

3.8. Correlation analysis between MACC1 expression and tumor immune infiltration level

To explore the relationship between *MACC1* expression and immune infiltration, we further analyzed it with the online TIMER database in 33 cancer types. The results revealed that *MACC1* expression was significant correlated with the level of B cell infiltration in 21 types of cancer, and significantly associated with infiltrating levels of CD8$^+$ T cells in 19 types of cancer, CD4$^+$ T cells in 18 types, macrophages in 19 types, neutrophils in 19 types, and dendritic cells in 16 types (Figure 6, Figure S5).

According to the correlations between *MACC1* expression and the level of immune-infiltration in various types of cancer, we next selected certain types of cancer in which higher *MACC1* expression levels shown significantly worse prognoses according to the TCGA database, such as LGG. And our analysis of TIMER data showed that *MACC1* expression was positively relation with immune infiltration. For example, as shown in Figure 6, *MACC1* was positively associated with B cell (r = 0.278, P = 6.43e-10), CD8+ T cell (r = 0.336, P = 4.76e-14), CD4+T cell (r = 0.332, P = 9.95e-14), macrophages (r = 0.457, P = 9.49e-26), neutrophil (r = 0.447, P = 1.15e-24) and dendritic cell (r = 0.446, P = 1.22e-24) immune infiltration levels of LGG. In contrast, negative correlations with infiltrating levels of CD8+ T cell (r = -0.179, P = 5.23e-04), CD4 + T cell (r = -0.105, P = 4.39e-02), macrophages (r = -0.229, P = 8.49e-06), neutrophil (r = -0.147, P = 4.60e-03) and dendritic cell (r = -0.24, P= 2.98e-06) were observed in STAD (stomach adenocarcinoma), in which *MACC1* expression is associated with better prognosis. We also used TIMER algorithms to analyze this relationship (Figure S6A). Collectively, these results were in line with expectations and strongly suggested that *MACC1* plays a vital role in tumor immunity, especially in these tumors.

Additionally, we used XCELL algorithms to elucidate the correlation between *MACC1* and diverse infiltrating lymphocytes across 33 cancer types. In this analysis, 64 types of cells were involved using XCELL algorithms, including adaptive and innate immune cells, hematopoietic progenitor cells, epithelial cells, and extracellular matrix cells. As shown in Figure S6B, we found the relationship between *MACC1* expression and immune infiltration differed between cancer types. But the results are consistent with TIMER databases in most cancers.
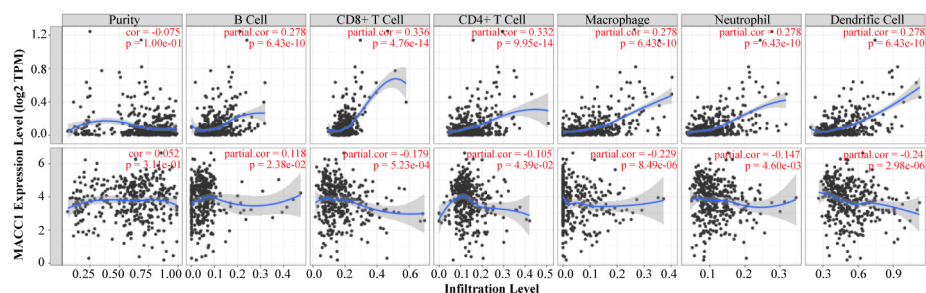

Figure 6. Correlation between *MACC1* and immune infiltration level.

The relationship between *MACC1* and six immune cells (B-cells, CD4$^+$T-cells, CD8$^+$T-cells, macrophages, neutrophils, and dendritic cells) in LGG and STAD.

3.9. MACC1 expression were correlated with immune cell type markers

Given that *MACC1* expression was found to be associated with immune cell infiltration, we investigated the relationship between *MACC1* expression and the representative immune markers of several immune cells, including B cells, CD8$^+$ T cells, T cells (general), macrophages, M1 macrophages, M2 macrophages, tumor-associated macrophages, monocytes, natural killer cells, neutrophils, dendritic cell, and diverse functional T cells. Expression levels of *MACC1* were found to be strongly correlated with the most immune markers. In LGG, 41 types of immune markers were positively correlated with *MACC1* expression, and in STAD, 30 types of immune markers showed negative correlations of *MACC1*, consistent with the above results (Table 2). The same results were observed after the adjustment for tumor purity and age. Taken together, the patient with higher *MACC1* expression levels may have a poorer prognosis and a significant positive correlation with immune infiltration and most sets of immune markers. Specifically, the results revealed that CD86, CCL2, IL10 of TAMs, CD14 of Monocyte, CD163, VSIG4, MS4A4A of M2 have significant associations with *MACC1* expression in LGG and STAD (P < 0.001). Most importantly, we found a significant correlation between *MACC1* expression and the regulation of several markers of T cells, such as CD8A of CD8$^+$Tcell, CD3D, CD3E, CD2 of T cell (general), CCR10, AHR of Th22, was observed in LGG and STAD. These results suggested that there was a potential mechanism where *MACC1* may regulate T cell functions. Meanwhile, it worth noting that several makers of Treg and T cell exhaustion were closely related to *MACC1* expressions, such as STAT5B, CCR8, TGFβ1, CTLA4, LAG3, GZMB. These immune marker genes played a key role in T cell exhaustion, indicating that *MACC1* may be involved in the immune escape.

Table 2. Correlation between MACC1 expression and immune cell type marker.

Cell type	Gene marker	LGG						STAD					
		None		purity		age		none		purity		age	
		cor	p	cor	p	cor	p	cor	p	cor	p	cor	p
	CD19	0.3000	***	0.2731	***	0.3308	***	-0.2446	***	-0.2210	***	-0.2344	***
B cell	CD38	-0.1206	**	-0.1334	**	-0.0614	0.1650	-0.1218	*	-0.1125	*	-0.1314	**
	CD79A	0.2047	***	0.2286	***	0.2361	***	-0.2327	***	-0.2031	***	-0.2319	***
CD8+Tcell	CD8A	0.3461	***	0.3538	***	0.3323	***	-0.1703	***	-0.1571	**	-0.1673	***
	CD8B	0.2227	***	0.2247	***	0.2090	***	-0.2275	***	-0.2373	***	-0.2213	***
	CD3D	0.5183	***	0.5214	***	0.5079	***	-0.1984	***	-0.1814	***	-0.1983	***
T cell (general)	CD3E	0.5515	***	0.5604	***	0.5402	***	-0.1893	***	-0.1697	***	-0.1884	***
	CD2	0.5662	***	0.5709	***	0.5525	***	-0.1619	***	-0.1397	**	-0.1647	***
	CD80	0.4756	***	0.4671	***	0.4877	***	0.0169	0.7309	0.0371	0.4719	-0.0046	0.9257
TAM	CD86	0.4007	***	0.3920	***	0.4453	***	-0.1517	**	-0.1464	**	-0.1633	***
	CCL2	0.3665	***	0.3644	***	0.3858	***	-0.2900	***	-0.3056	***	-0.2811	***
	IL10	0.3796	***	0.3629	***	0.4065	***	-0.0967	*	-0.1058	*	-0.1062	*
	CXCR5	0.2636	***	0.2575	***	0.2734	***	-0.1894	***	-0.1612	**	-0.1727	***
Tfh	ICOS	0.5196	***	0.5134	***	0.5120	***	-0.0699	0.1553	-0.0359	0.4854	-0.0851	0.0873
	BCL6	-0.0110	0.8024	-0.0276	0.5473	0.0273	0.5371	-0.2195	***	-0.2198	***	-0.2121	***

Cell type	Gene marker	LGG						STAD					
		None		purity		age		none		purity		age	
		cor	p	cor	p	cor	p	cor	p	cor	p	cor	p
Th1	STAT1	0.4891	***	0.4811	***	0.4764	***	0.0860	0.0801	0.1013	*	0.0809	0.1041
	IFNG	0.2944	***	0.2919	***	0.2905	***	-0.0324	0.5105	-0.0079	0.8785	-0.0478	0.3377
	CCR3	0.3355	***	0.3410	***	0.3375	***	-0.0348	0.4800	-0.0143	0.7809	-0.0410	0.4111
Th2	GATA3	0.5002	***	0.5044	***	0.4711	***	-0.1644	***	-0.1553	**	-0.1607	**
	STAT5A	0.4410	***	0.4281	***	0.4601	***	0.0104	0.8324	0.0189	0.7145	0.0034	0.9461
Th9	TGFBR2	0.4199	***	0.4017	***	0.4561	***	0.0672	0.1718	0.0622	0.2273	0.0781	0.1168
	IRF4	0.1375	***	0.1175	***	0.1855	***	-0.1395	***	-0.1135	**	-0.1550	***
Th17	IL21R	0.1629	***	0.1686	***	0.1717	***	-0.1651	***	-0.1387	**	-0.1696	***
	STAT3	0.4223	***	0.4037	***	0.4368	***	0.0377	0.4438	0.0411	0.4253	0.0229	0.6456
Th22	CCR10	0.2110	***	0.2097	***	0.1851	***	-0.2561	***	-0.2566	***	-0.2481	***
	AHR	0.3579	***	0.3476	***	0.4009	***	0.3871	***	0.3893	***	0.3783	***
Treg	CCR8	0.2271	***	0.2324	***	0.2116	***	-0.0113	0.8190	-0.0006	0.9914	-0.0193	0.6987
	STAT5B	-0.0385	0.3833	-0.0326	0.4767	-0.0150	0.7343	0.0207	0.6746	0.0162	0.7536	0.0218	0.6623
	TGFB1	0.3335	***	0.3140	***	0.3851	***	-0.1751	***	-0.1639	**	-0.1820	***
T cell (exhaustion)	CTLA4	0.3479	***	0.3383	***	0.3469	***	-0.1141	*	-0.0915	0.0751	-0.1315	**
	LAG3	0.2768	***	0.2954	***	0.2914	***	-0.1936	***	-0.1853	***	-0.2056	***
	GZMB	0.3564	***	0.3813	***	0.3255	***	-0.1001	*	-0.0799	0.1206	-0.1170	*
	XCL1	0.4235	***	0.4168	***	0.4331	***	-0.2295	***	-0.2335	***	-0.2277	***
NK	KIR3DL1	0.1350	***	0.1403	***	0.1393	***	-0.1605	***	-0.1570	***	-0.1715	***
	CD7	0.5055	***	0.5146	***	0.5177	***	-0.2073	***	-0.1951	***	-0.2199	***
Monocyte	CD14	0.3595	***	0.3379	***	0.4062	***	-0.1944	***	-0.2080	***	-0.1967	***
Macrophage	CD68	0.4314	***	0.4167	***	0.4650	***	0.0576	0.2415	0.0596	0.2473	0.0493	0.3221
M1	NOS2	-0.0084	0.8491	-0.0129	0.7786	-0.0245	0.5800	0.2512	***	0.2618	***	0.2277	***
	I5	0.3861	***	0.3806	***	0.4182	***	0.0122	0.8042	0.0107	0.8351	0.0195	0.6961
M2	CD163	0.5021	***	0.4900	***	0.5125	***	-0.0821	0.0950	-0.0853	0.0972	-0.1056	*
	VSIG4	0.2379	***	0.2028	***	0.2878	***	-0.1702	***	-0.1820	***	-0.1758	***
	MS4A4A	0.4084	***	0.3912	***	0.4383	***	-0.1663	***	-0.1762	***	-0.1705	***
Neutrophil	MPO	0.0085	0.8481	0.0009	0.9841	-0.0446	0.3129	-0.1189	*	-0.1039	*	-0.1216	*
	CCR7	0.3712	***	0.3739	***	0.3610	***	-0.1862	***	-0.1691	***	-0.1792	***
DC	CD1C	0.3017	***	0.2972	***	0.3212	***	-0.1579	**	-0.1580	**	-0.1367	**

*P < 0.05, **P <0.01, ***P < 0.001.

Tfh, follicular helper T cell; Th, T helper cell; Treg, regulatory T cell; TAM, tumor associated macrophage; NK, natural killer cell; DC, dendritic cell; None, correlation without adjustment; Purity, correlation adjusted for tumor purity; Cor, R value of Spearman's correlation.

3.10. The relationship between MACC1 copy number variations and immune infiltrates

Here we analyzed genetic alterations in *MACC1* in different tumor samples from TCGA cohort. The results revealed the main type of genetic alteration of *MACC1* were missense mutation and amplification. And the highest frequency of *MACC1* alteration (＞8%) occurred in patients with melanoma tumors, in which "mutation" was the predominant type of alteration. The "amplification"

was the dominant type in people with esophageal squamous cell carcinoma, sarcoma, seminoma, pancreatic adenocarcinoma, and pheochromocytoma (Figure 7A).

We also investigated genetic alteration of *MACC1* across different cancers. Furthermore, we analyzed whether the *MACC1* copy number variations impact the level of immune infiltrates, including six types of immune cells. As shown in Figure 7B, deletion and amplification of *MACC1* were both found to be correlated with the lower levels of immune infiltrates in STAD and LUSC. In SKCM and HNSC, lower levels of six immune cells are mainly associated with the amplification of *MACC1*. Conversely, in LUAD and HNSC-HPV neg, deletion of *MACC1* was related to the same results.
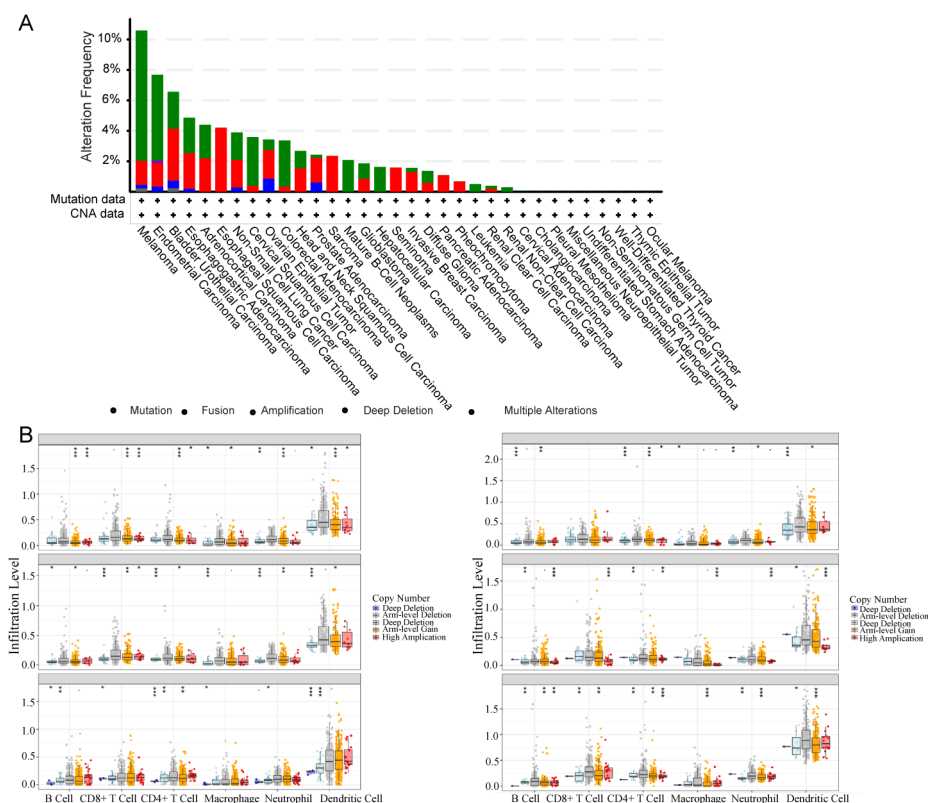

Figure 7. Mutation feature of *MACC1* across different cancer and correlation between MACC1 copy number variations and immune infiltration. (A) The mutation features of *MACC1* across pan-cancer in TCGA was explored using the cBioPortal tool. The results are displayed according to cancer type detailed. (B)The association between *MACC1* copy number variations and immune infiltrates in LUSC (lung squamous cell carcinoma), SKCM (skin cutaneous melanoma), STAD (stomach adenocarcinoma), HNSC (head and neck cancer), HNSC-HPV neg (head and neck cancer-HPV negative), LUAD (lung adenocarcinoma). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

4. Discussion

To our knowledge, this is the first pan-cancer analysis of *MACC1*, providing novel insights into expression pattern, function, association with immune cell infiltration, and disease prognosis, which together form a solid basis for further research into diagnostic and therapeutic strategies. And the results of our analysis can help to prioritize cancer types for further clinical trials and experimental studies.

Metastasis-associated in colon cancer-1 (*MACC1*) was first identified through genome-wide expression analyses in 2009. Furthermore, they also revealed that *MACC1* can induce cell proliferation, invasion, migration, and regulate transcription of genes involved in the metastasis process [5]. Jan et al. demonstrated that *MACC1* was significantly correlation with the key transcription genes in more than half of the cancer types in the big data analysis, including receptor tyrosine kinase MET, hepatocyte growth factor (HGF) and recombinant Matrix Metalloproteinase 7 (MMP7) [25]. In addition, the meta-analysis results suggested *MACC1* was correlation with regional invasion and distant metastasis [26,27]. *MACC1* is a significant biomarker for the prognosis of colorectal cancer, but it is also applicable to various other forms of solid cancer. It has been proved to be a predictor of many solid cancers, such as lung, breast, and pancreatic cancer. Pan-cancer analysis displays the similarities and differences between genomic and cellular changes in different tumor types and provides new insights into cancer prevention and treatment targets [28]. However, the role of *MACC1* in a full spectrum of tumors has not been identified.

According to our gene homologous and phylogenetic tree analysis in our study, the protein structure of *MACC1* was found to be conserved, indicating that the normal physiological function of *MACC1* may be similar among species. This provides new ideas for understanding the role in the pathogenesis of different tumors.

In this study, *MACC1* was differentially expressed in tumor versus normal tissues, and its expression varied across diverse cancer types. These results suggest that *MACC1* may play different roles in different cancers. Nevertheless, this was a preliminary assumption. To verify the role of *MACC1* in tumors, we performed prognostic analyses in cancers with different *MACC1* expression levels, which revealed that higher *MACC1* expression was correlated with better prognoses in KIRC, SKCM, KIRP, but worse prognoses in COAD, LGG, UVM, PAAD. Our Kaplan-Meier plotter analyses showed that high level of *MACC1* expression was correlated with better prognosis in lung and gastric cancer, but poor prognosis in ovarian cancer. In addition, wang et al demonstrated that high expression of *MACC1* predicts a worse prognosis of gynecologic cancers and breast cancer. But further studies with larger sample sizes are needed to validate the role of *MACC1* in gynecologic cancers [29]. As a predictor for metastasis formation in colon cancer, we future investigated the relationship between clinical phenotypes and the expression level of *MACC1*. A previous study examined the expression of *MACC1* in 52 clinical specimens with different pathological stages in colorectal carcinoma using qRT-PCR [30]. The results are consistent with ours, *MACC1* expression was higher in patients with advanced colon cancer. The trend was also observed in CESE, ESCA, UCEC, THCA; however, *MACC1* expression was higher in the early-stage KIRP, KIRC, and KICH compared with later stage disease. These results suggest that the role of *MACC1* varies between cancer types, and may serve as an indicator of disease progression in some cancers and as an early warning in others. Cancer is a highly heterogeneous set of disease; emerging evidence suggests that differences in signal transduction driven by the same oncogene may lead to different clinical outcomes depending on the tumor type, which may help to explain our findings.[31] For example, the BRAF inhibitor is effective in people with melanoma who have a BRAF-driven mutation, but not in those with colorectal cancer [32,33]. Gene function may vary between different cancers and genetic changes in the same cancer may have different outcomes. Our results provide evidence for *MACC1* as an independent prognostic factor, but that the underlying mechanisms for its role and prognostic value in different cancer types require further study.

The transformation of normal cells into cancer cell mostly involves proteomics, transcriptomics, epigenetics, and somatic acquired genetic changes [34]. Epigenetic modification within the genome, including methylation, can affect disease progression. A previous study demonstrated that hypomethylation can cause genome instability and lead to gene activation [35]. An important characteristic of many tumors is the hypomethylation of oncogene promoters, and hypermethylation of tumor suppressor gene promoters. Abnormal DNA methylation can be used as an important molecular marker for tumor diagnosis, classification, and treatment [36,37]. And we found that *MACC1* expression varies across cancer types and is significantly correlated with DNA methylation status [38]. Previous studies show that demethylation may activate the expression of the tumor genes, resulting in up-regulation or instability in the chromosomal structure. Hypermethylation may affects the cell cycle by silencing genes resulting in down-regulation and inhibition of DNA damage repair [37]. Most cancer types in this study were associated with a high expression level of *MACC1* and corresponding low methylation levels of *MACC1* promoters compared with normal tissue, supporting both previous research and the role of *MACC1* as a potential biomarker. In our study, we have confirmed this theory. In most cancers, with a high expression level of *MACC1*, the methylation levels of *MACC1* promoters are lower than in normal tissue. Above all, we can speculate that there is a certain relationship between increased *MACC1* expression levels and methylation in cancer, suggesting that *MACC1* is an attempt to identify the role of biomarkers.

Immunotherapy is an important treatment option for people with cancer these years. However, only a small proportion of people benefit from immune checkpoint inhibitors. Therefore, the exploration of promising biomarkers is a clinical priority. Whether there is a relationship between *MACC1* and immunity has attracted our attention. The adaptive immune system can recognize cancer by non-autoantigens associated with somatic mutations. TMB levels influence the generation of immunogenic peptides, which in turn affect the patient's response to immune checkpoint inhibitors. And genomic instability is a new feature of cancer and is closely related to cancer development, progression and metastasis [39]. Microsatellite instability (MSI) level can affect TMB level, which is a crucial index to predict tumorigenesis. And both TMB and MSI can be used to predict the potential efficacy of immunotherapy. It is important to explore the relationship between *MACC1* expression and TMB levels in cancer patients. In our study, an association between TMB and *MACC1* was observed in 14 types of cancer and between MSI and *MACC1* in six types of cancer. A previous study found that *MACC1* works with mismatch repair (MMR) to predict the response to adjuvant chemotherapy. And *MACC1* stratifies colon cancer patients with poor pMMR status and tumor patients with pMMR/*MACC1*-low tumors have similar outcomes as dMMR patients. This further suggests a potential functional link between *MACC1* and genomic instability [40].

Importantly, we further explored the relationship between immune infiltration and *MACC1* expression in selected cancers based on our prognostic results. The tumor microenvironment (TME) has been the focus of recent oncology research, which is mainly is mainly composed of tumor-infiltrating lymphocytes (TILs; B cells and T cells), dendritic cells, neutrophils, and macrophages [41,42]. Immune cells, as a double-edged sword, play a role in promoting or suppressing cancer in different situations [43]. On the one hand, the tumor microenvironment not only contributes to tumor genesis, development, metastasis, and tumor immune tolerance. Extensive researches have proved that tumor-associated macrophages and other inflammatory cells can promote angiogenesis and many tumor cells can evade the surveillance of immune cells through a variety of mechanisms. On the other hand, some inflammatory cells, notably T cells, have inhibitory properties, monitoring and destroying tumor

cells and are associated with a favorable prognosis in many tumor types [44]. In our study, we found that *MACC1* expression were inversely correlated with immune infiltration (especially, the CD4$^+$ T cell and CD8$^+$ T cell, macrophages, neutrophils, dendritic cells), in which *MACC1* expression is related to better prognosis. In contrast, in other cancer types, the level of immune infiltration was positively correlated with *MACC1* expression. These results revealed a strong correlation between *MACC1* and immune infiltration, but that this relationship varied between cancer types. However, *MACC1* may lead to tumorigenesis or inhibit tumor progression by changing the state of tumor-infiltrating lymphocytes. As an initial investigation of the potential mechanism underlying this process, the correlation between *MACC1* expression and that of immune cell markers was further assessed. First, gene markers of TAMs such as CCL2, CD86, and IL-10 showed correlations with *MACC1* expression. Meanwhile, M2 macrophage markers such as VSIG4 and MS4A4A were strongly related to *MACC1* expression. These results demonstrated that *MACC1* expression may be related to the polarization of tumor-associated macrophages. Moreover, another interesting aspect of our study is that the potential regulating role of *MACC1* in T cell function. T cells are an important component of adaptive immunity and have become a focus of immunotherapy owing to their capacity for specific antigen recognition and tumor destruction [45]. In our study, a significant correlation can be observed between *MACC1* expression and T helper cells (Th22), T cell (general). As we all know, the immune system prevents tumor progression mainly by activating T cells and macrophages. However, as the tumor progresses, the immune infiltration will transform to promote tumor progression and inhibit immune cell-mediated cytotoxicity. Identifying and characterizing interactions between the immune system and the tumor is crucial for both reducing drug resistance and developing new cancer treatments. These results may provide new insights into the tumorigenesis of *MACC1* and immune infiltration; however, further research is needed to clarify the exact mechanism for this. In addition, our analysis of some common immune checkpoints, such as SIGLEC15, TIGIT, CD274, HAVCR2，PDCD1，CTLA4，LAG3, and PDCD1LG2, demonstrated that these were associated with most of the cancers; Tong and his colleagues verified that *MACC1* can regulate PDL1 expression in gastric cancer cells. And the positive results suggest that *MACC1* may be a therapeutic target for immunotherapy [46]. In particular, SIGLEC15 may be a potential treatment option for patients who are not likely to benefit from anti-PD1 therapy [47]. Overall, our study confirmed the predictive value of *MACC1* as a metastatic biomarker. And alterations in the expression of *MACC1* may promote tumor progression by influencing the levels of various cancer marker genes; this process could potentially be prevented by targeting the expression or function of *MACC1* as a therapeutic strategy, limiting or even preventing metastasis.

Although in our pan-cancer analysis, the role of *MACC1* expression has been extensively explored in various genetic and biological processes, including methylation, prognosis, immune infiltration, etc.; however, there are some limitations. First, multiple analyses based on diverse databases can provide large amounts of data. But the discrepancies in results may be a reflection in data collection approaches, data heterogeneous to a certain extent, and underlying mechanisms pertinent to different biological properties. Second, our analysis is based on various databases, and interpretation of our analysis results is inherently limited by this approach. Therefore, experimental studies are needed to elucidate the mechanism of action of *MACC1* and further clinical trials are needed to validate possible therapeutic interventions in the process of transition into the clinic. In the future, there will be more researches to validate the highlights of our bioinformatics analysis.

5. Conclusions

In conclusion, our pan-cancer analysis for the first time revealed that the expression of *MACC1* was statistically significant with clinical prognosis, pathological stage, immune infiltration, DNA methylation, cell infiltration of multiple tumors, and drug targets, etc., contributing to a more comprehensive understanding of the role of *MACC1* in tumorigenesis, and the identification of potential therapeutic targets. More importantly, we explored that *MACC1* may closely related to cancer immunity.

Acknowledgments

Conflict of interest

The authors declare that they have no competing interests.

Data availability statement

The study analyzed publicly available data sets, which can be found in The Cancer Genome Atlas (https://portal.gdc.cancer.gov), GEO database (https://www.ncbi.nlm.nih.gov/geo), cbioportal (http://www.cbioportal.org), Oncomine database (https://www.oncomine.org/resource/main.html).

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