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CCL5 might be a prognostic biomarker and associated with immuno-therapeutic efficacy in cancers: A pan-cancer analysis

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ABSTRACT

Purpose: Chemokine ligand 5 (CCL5), a vital member of the CC chemokine family, plays diverse roles in tumorigenesis, metastasis, and prognosis in various human tumors. However, no pan-cancer analysis has been conducted to illustrate its distinctive effects on clinical prognosis via underlying mechanisms and biological characteristics.

Methods: Herein, we exploited the existed public bioinformatics database, primarily TCGA database and GTEx data, to comprehensively analyze the value of CCL5 involved in patient prognosis.

Results: This study found that CCL5 was excessively expressed in most tumors and significantly associated with clinical prognosis in 10 out of 33 types of tumors. Notably, CCL5 might be an independent predictive biomarker of clinical outcome in SKCM patients, confirmed by univariate and multivariate Cox regression analysis. Furthermore, we acquired the genetic alteration status of CCL5 in multiple types of tumor tissues from TCGA cohorts. We revealed a potential correlation between the expression level of CCL5 and tumor mutational burden in 33 types of tumors. In addition, data showed that DNA methylation was associated with CCL5 gene expression in THCA, PRAD, LUSC, and BRCA cancers. Immune infiltration and immune checkpoints are fine indexes for evaluating immunotherapy. We uncovered that CCL5 was negatively correlated with the immune infiltration of $CD8^+$ T cell, $CD4^+$ T cell, macrophages, and gamma delta T cells in BRCA-basal and CESC tumors, while a significant positive correlation was observed in BLCA, COAD and other 7 types of tumors. Besides, CCL5 was closely associated with the immune checkpoint molecules in 8 types of tumors. The TIDE score was less in the CCL5 high-expressed group than in the CCL5 low-expressed group in SKCM patients, which indicated that CCL5 might be a fine monitor of immune response for immunotherapy. GO enrichment analysis data uncovered that cytokine-cytokine receptor interaction and chemokine signaling might be involved in the role of CCL5 in regulating tumor pathogenesis and prognosis.

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Conclusion: In conclusion, CCL5 was preliminarly identified as a biomarker of immune response and prognosis for tumors patients via our first comprehensive pan-cancer analysis.

1. Introduction

The Cancer Genome Atlas (TCGA) is a public database for exploring and revealing the key oncogenes alterations according to filed tumors information. Pan-cancer analysis was initiated and conducted by the TCGA researchers in 2012, which is a new comprehensive analysis of various tumors' biological characteristics and clinical prognosis [1, 2, 3]. Considering the complexity of oncogenesis and tricky of tumor treatment responding, it's crucial to conduct a pan-cancer analysis to uncover key interested genes and investigate their potential contributions to clinical outcomes.

Emerging reports considered that pan-cancer analysis is beneficial for revealing potential mechanisms of tumorigenesis and clinical prognosis. In recent years, significant progress has been made in cancer immunotherapy (CIT) due to its curable effect on various tumors, interfering with tumor immune evasion [4]. The immune response is a sensitive index reflecting patients' clinical outcome in CIT, and that tumor mutation burden (TMB), deep sequencing of T -cell receptor DNA, PD-L1 expression and eosinophilic count were considered available indicators for immune response evaluation [4, 5, 6]. However, a recent study manifested that the predictive efficacy of reflecting immune response by the biomarkers mentioned above was poor, because of many patients being assessed as nonresponsive or responsive feebly, even those who acquired good outcomes in CIT [5, 7]. Therefore, we must explore more efficacious biomarkers for defining whether patients are suited for CIT with high performance.

Chemokine ligand 5 (CCL5), a vital member of the CC chemokine family, is secreted by platelets, synovial fibroblasts, T lymphocytes, macrophages, tubular epithelium, and even tumor cells [8]. It has also known as RANTES, with strong chemotaxis of monocytes and T lymphocytes, which is the principal mechanism of CCL5-mediated chronic inflammatory diseases and cancers [9]. A large of studies confirmed that CCL5 was highly expressed in many tumors, including hematological malignancies [10, 11], breast cancer [12, 13], prostate cancer [14], hepatocellular carcinoma [15], and glioblastoma [16], which indicated a high level of CCL5 was associated with tumor progression, invasion, and metastasis. However, other reports showed that CCL5 recruited dendritic cells and antitumor T cells to the tumor microenvironment, thereby enhancing immunotherapy reactivity in tumors [17, 18, 19, 20]. Thus, CCL5 is considered a two-edged sword in tumor biology, and the detailed mechanism of its roles in tumors needs to be elucidated urgently.

In this study, we performed a comprehensive pan-cancer analysis of CCL5, using TCGA, CPTAC, and other public databases to uncover potential molecular mechanisms of CCL5 contributing to tumorigenesis and clinical prognosis in different types of tumors. Many parameters were utilized in our study, including gene expression, genetic alterations, DNA methylation, clinical prognosis, and immune infiltration information to elicit a comprehensive and excellent illustration of CCL5's role in tumor biology. Our study may provide a relatively overall understanding of the roles of CCL5 in various tumors.

2. Materials and methods

2.1. Gene expression analysis

We analyzed data on CCL5 of 33 tumor types from the TCGA database in the web of home for researchers (https://www.home-for-researchers.com/). Most tumors had CCL5 expression information compared with adjacent normal tissue samples. Then, these data were utilized for analyzing the discrepancy of CCL5 expression between tumors and normal tissues in different specific tumor subtypes or tumors. The significant difference was defined by $P < 0.05$.

2.2. Protein expression analysis

We analyzed protein expression using the Clinical Proteomic Tumor Analysis Consortium (CPTAC) dataset in the UALCAN portal (http://ualcan.path.uab.edu/index.html), and compared total CCL5 protein between normal tissues and primary tumors by entering "CCL5". Unexpectedly, only six acquired types of tumor information are available, including lung adenocarcinoma, breast cancer, endometrial carcinoma, ovarian cancer, colon cancer, and clear cell renal cell carcinoma.

2.3. Survival analysis

We employed the "Survival Map" module of GEPIA2 to obtain the overall survival (OS) and disease-free survival (DFS) significance map of CCL5 from 33 types of tumors (http://gepia2.cancer-pku.cn/#index). Threshold values were set to distinguish high-expression and low-expression groups by cutoff-high (50%) and cutoff-low (50%). Furthermore, we obtained survival plots by using the "Survival Analysis" module in the GEPIA2 web and utilized a log-rank test to detect the statistical significance of survival analysis.

2.4. Genetic alteration analysis

We selected the "TCGA Pan-Cancer Atlas Studies" in the "Quick select" section of the cBioPortal web (http://www.cbioportal.org/)

and entered the search terms "CCL5" to obtain the genetic alteration characteristics of CCL5. Alteration frequency, mutation type, and copy number alteration (CNA) information contained 33 types of tumors were achieved via the "Cancer Types Summary" module. The "Comparison" module was employed to calculate the overall disease-free, progression-free, and disease-free survival of these cases with or without CCL5 genetic alteration. Besides, Kaplan–Meier plots with log-rank P-values were generated.

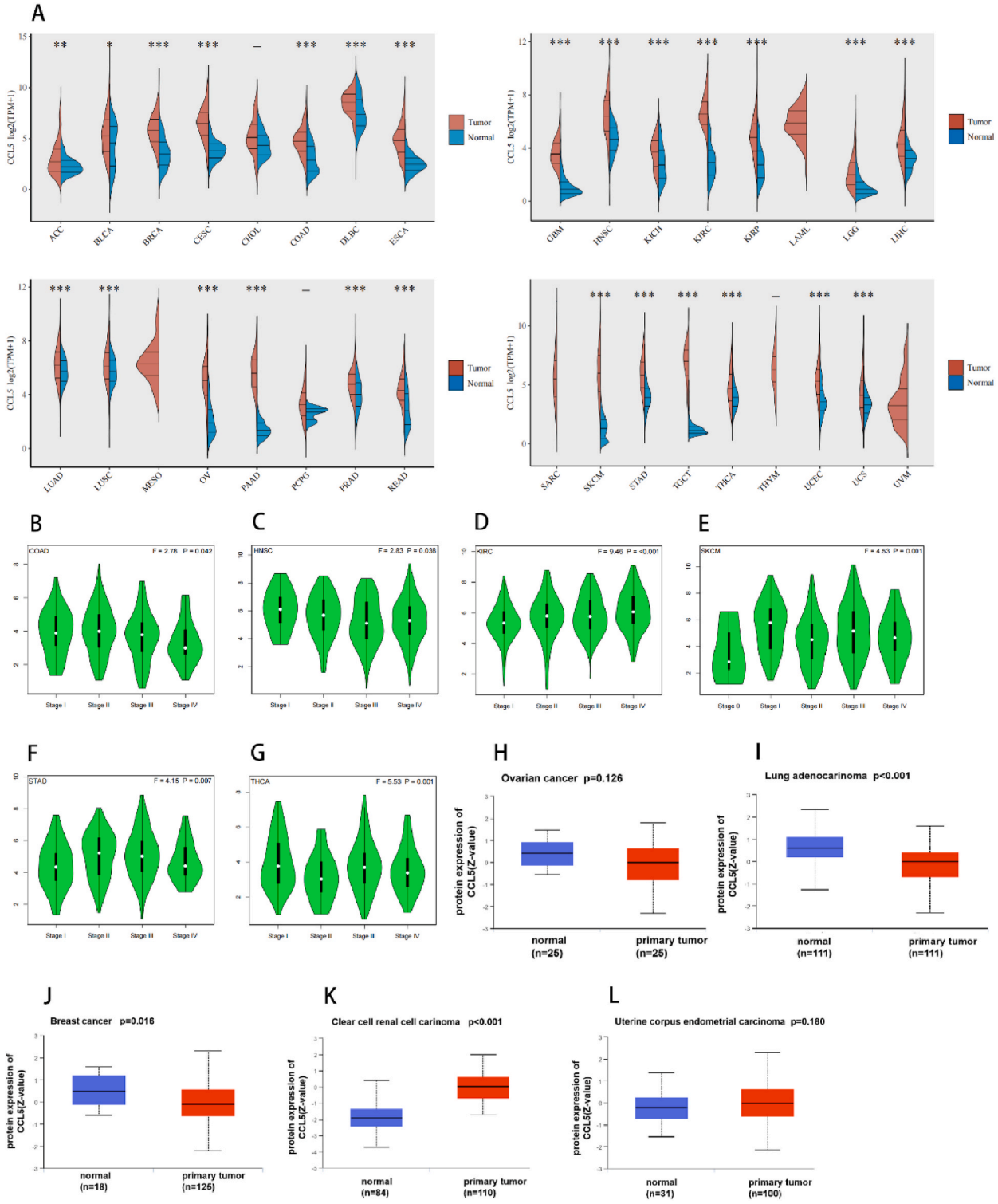

Figure 1. Expression level of CCL5 in different tumors.

2.5. Immune infiltration analysis

We applied the "Immune-Gene" module of the TIMER2 web (http://timer.cistrome.org/) to investigate the potential correlation between CCL5 expression and immune infiltration in all TCGA documented tumors. This procedure assigned CD8+ T cells, macrophages, CD4+ T cells, and gamma delta T cells for immune infiltration components. MCPCOUNTER, TIMER, QUANTISEQ CIBERSORT, XCELL, CIBERSORT-ABS, and EPIC algorithms were employed to analyze immune checkpoints and estimate immune infiltration scores. The purity-adjusted Spearman's rank correlation test obtained partial correlation and P-values. In the end, a heatmap was selected to present the result data.

2.6. CCL5-related gene enrichment analysis

To uncover proteins associated with CCL5, we input the terms "Homo sapiens" and "CCL5" and set the main parameters in the STRING website (https://string-db.org/) displayed in Supplement Table S1. Eventually, we acquired CCL5-binding proteins screened from former experimental testified. The "Similar Gene Detection" module in the GEPIA2 web was applied to obtain the top 100 CCL5-

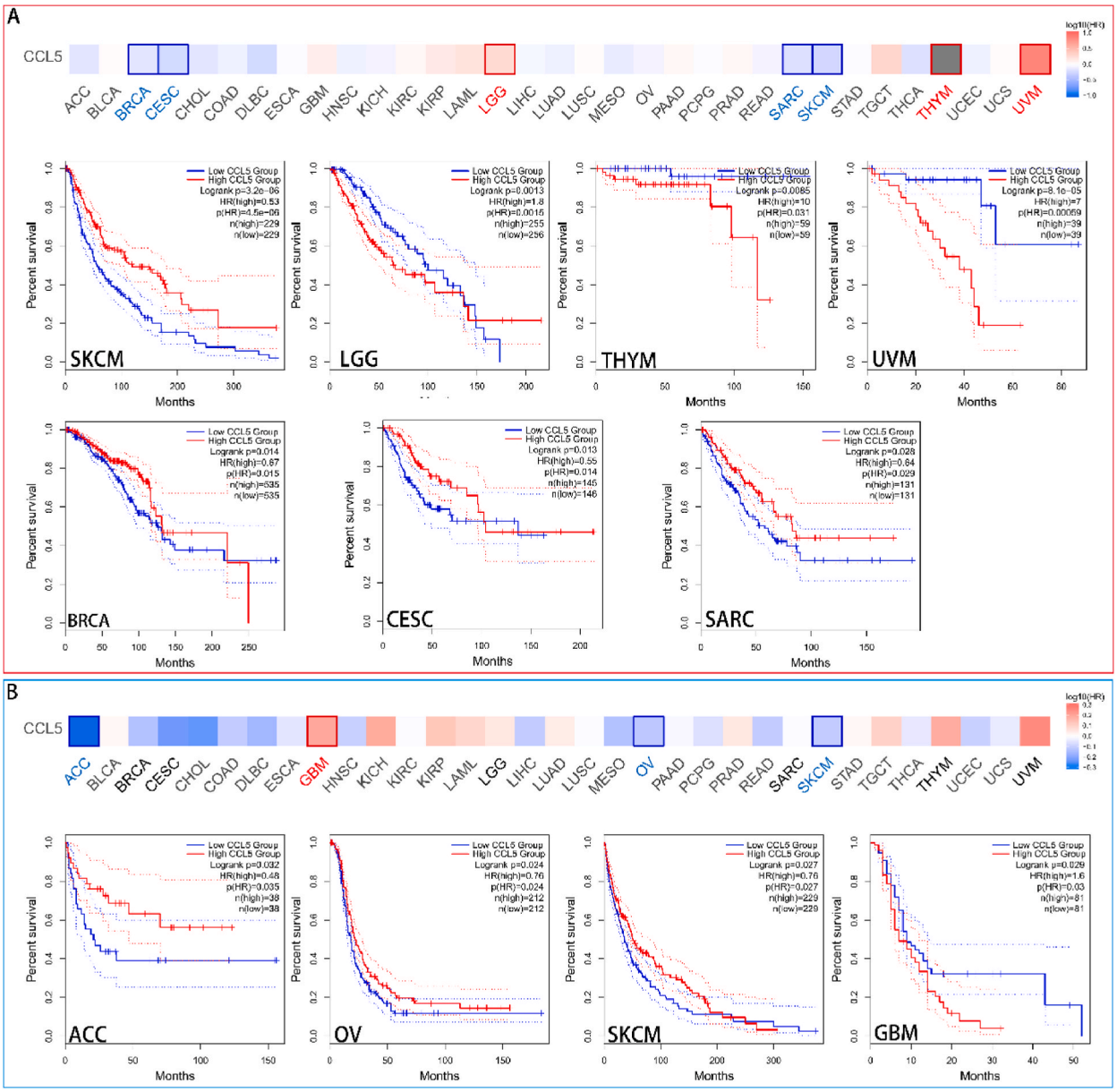

Figure 2. The potential relationships between CCL5 expression and survival rates of patients.

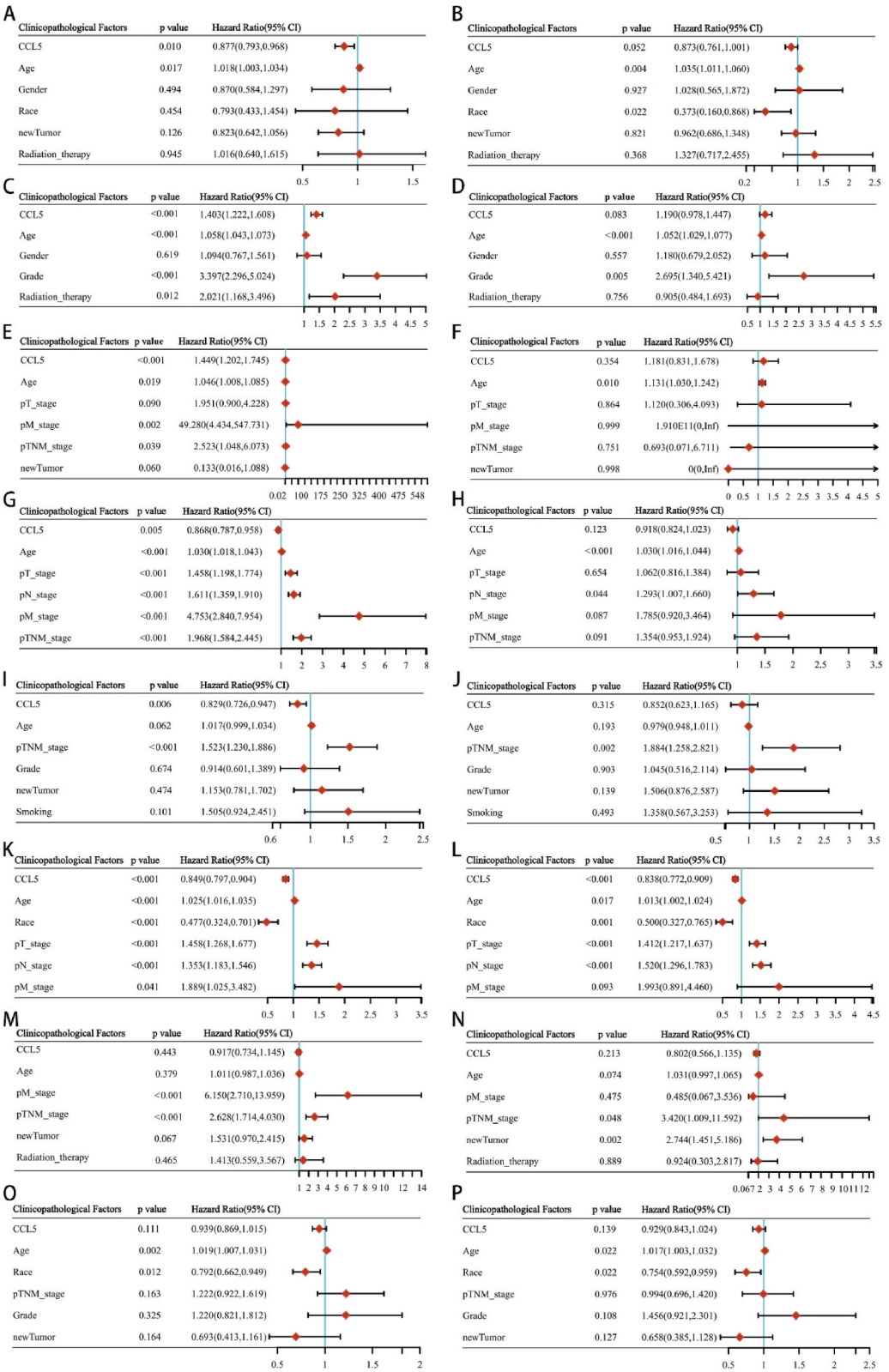

Figure 3. CCL5 was associated with prognosis in various tumors and as an independent prognostic factor for SKCM.

correlated target genes from normal tissues and tumors in the TCGA dataset. In addition, the "Correlation Analysis" module was utilized to conduct a pairwise Pearson's correlation between these targeted genes and CCL5. Besides, the "Gene Cor" module of TIMER2 was employed to investigate correlation and P value within heatmap displayed genes. Jvenn, an interactive Venn diagram viewer, was applied to calculate these intersected genes from CCL5 associated with and correlated.

Furthermore, we collected these intersected genes (the top 100 CCL5-correlated target genes and the genes of the target binding proteins) and conducted a KEGG pathway analysis. The gene list was uploaded to the KEGG database for annotation, visualization, and calculation with parameters set in Supplement Table S1. Enrichment pathways were visualized by the "tidyr" and "ggplot2" packages in R software. In addition, we used the "cluster Profiler" package to conduct gene ontology (GO) enrichment analysis. Thus, these biological processes, cellular components, and molecular function data were presented. Statistically significant was defined by $P < 0.05$ (two-tailed).

3. Results

3.1. CCL5 was highly expressed in the vast majority of tumors

We utilized the TIMER2 approach and GTEx dataset to inquire about the CCL5 expression status from these various types of tumors in TGCA. As shown in Figure 1A, the mRNA level of CCL5 was highly expressed in 26 types of tumors out of 33 types, including BRCA (breast invasive carcinoma), GBM (glioblastoma multiforme), LUAD (lung adenocarcinoma), SKCM (skin cutaneous melanoma), etc. What is more, except for ACC (adrenocortical carcinoma, $P < 0.01$) and BLCA (bladder urothelial carcinoma, $P < 0.05$), another left 24 types of tumors expressed CCL5 significantly higher compared with their corresponding normal tissues ($P < 0.001$). However, we did not acquire a significant difference in PCPG (pheochromocytoma and paraganglioma) and CHOL (cholangiocarcinoma). Unexpectedly, there were five types of tumors, including LAML, MESO, SARC, THYM, and UVM, with no information on CCL5 expression in their corresponding normal tissues. Furthermore, we checked the alterable status of CCL5 expression companying with pathological stages. Data indicated that CCL5 was associated with pathological cancer stages in COAD, HNSC, KIRC, STAD, THCA, and SKCM in Fig.1(B-G). Other tumors with no significant change of CCL5 mRNA expression following their pathological stages.

CCL5 protein status in CPTAC datasets indicated that clear-cell renal cell carcinoma tissues expressed a significantly high level of CCL5 protein ($P < 0.001$) (Figure 1K), but ovarian cancer and uterine corpus endometrial carcinoma were not significantly different compared with their normal tissues (Fig.1H and Fig.1L). Unexpectedly, CCL5 protein in lung adenocarcinoma and breast cancer were significantly less than in their corresponding normal tissues ($P < 0.05$) (Fig.1I and Fig.1J). Besides that, no data on CCL5 protein information was available from other types of tumors in the CPTAC database.

1. The expression level of CCL5 gene in different cancers was analyzed in the TCGA and GTEx dataset (A). 2. Expression level of CCL5 gene in different pathological stages of COAD (B), HNSC (C), KIRC (D), SKCM (Figure 1E), STAD (F), and THCA (G). 3. The expression level of CCL5 protein between normal tissue and primary tissue in ovarian cancer (H), lung adenocarcinoma (I), breast cancer (J), clear cell renal cell carcinoma (K), and uterine corpus endometrial carcinoma (L). 4. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

3.2. CCL5 was associated with prognosis in various tumors and as an independent prognostic factor for SKCM

We divided tumor cases into high-expression and low-expression groups according to expression levels of CCL5. We calculated the correlation between the CCL5 expression and the prognosis of patients in 33 types of cancer, mainly utilizing TCGA and GEO databases. We observed that highly expressed CCL5 was associated with an unfavorable outcome of overall survival in LGG ($P = 0.001$), THYM ($P = 0.009$), and UVM ($P < 0.001$). Whereas, lowly expressed CCL5 was correlated with serious prognosis of overall survival in BRCA ($P = 0.014$), CESC ($P = 0.013$), SARC ($P = 0.028$) and SKCM ($P < 0.001$) (Figure 2A). In disease-free survival analysis, data showed that highly expressed CCL5 was significantly associated with worse prognosis in GBM ($P = 0.029$), however, lowly expressed CCL5 was inclined to poor disease-free survival prognosis in ACC ($P = 0.032$), OV ($P = 0.024$), and SKCM ($P = 0.027$) (Figure 2B).

Due to the expression level of CCL5 being associated with OS or/and DFS in the mentioned above types of tumors, we conducted univariate and multivariate Cox regression analyses to investigate whether CCL5 may be an independent prognostic factor in these patients. In SARC patients, data indicated that age, but not CCL5, was significantly associated with OS acquired from multivariate Cox regression analysis (Fig.3A and Fig.3B); it means age could be an independent prognostic factor for SARC patients. In LGG patients, Age and Grade have been linked to OS resulting from multivariate Cox regression analysis, revealing they could be an independent prognostic factor in LGG patients (Fig.3C and Fig.3D). Unexpectedly, data indicated that the expression level of the CCL5 was not a candidate independent prognostic factor of OS in SARC (Fig.3A and Fig.3B), LGG (Fig.3C and Fig.3D), UVM (Fig.3E and Fig.3F), BRCA (Fig.3G and Fig.3H), CESC (Fig.3I and Fig.3J), ACC (Fig.3M and Fig.3N), and OV patients (Fig.3O and Fig.3P). Nevertheless, CCL5, Race, pT_stage and pN_stage, and age were significantly associated with the overall survival in SKCM patients, resulting from both univariate and multivariate Cox regression analyses, which manifested that CCL5 could be an acceptable independent prognostic factor for SKCM patients (Fig.3K and Fig.3L).

We used the GEPIA2 tool to perform overall survival (A) and disease-free survival (B) analyses of different tumors in TCGA by CCL5 gene expression. The survival map and Kaplan-Meier curves with positive results displayed in figure A and B respectively.

Univariate (A) and multivariate (B) Cox regression of prognosis indicators for OS of SARC patients. Univariate (C) and multivariate (D) Cox regression of prognosis indicators for OS of LGG patients. Univariate (E) and multivariate (F) Cox regression of prognosis indicators for OS of UVM patients. Univariate (G) and multivariate (H) Cox regression of prognosis indicators for OS of BRCA patients. Univariate (I) and multivariate (J) Cox regression of prognosis indicators for OS of CESC patients. Univariate (K) and multivariate (L)

Cox regression of prognosis indicators for OS of SKCM patients. Univariate (M) and multivariate (N) Cox regression of prognosis indicators for OS of ACC patients. Univariate (O) and multivariate (P) Cox regression of prognosis indicators for OS of OV patients.

3.3. Genetic alteration of CCL5 existed in various tumors

We observed the genetic alteration status of CCL5 in various types of tumor tissues from TCGA cohorts. The "amplification" alteration resulted from copy number variation in the genome, which almost exclusively occurred in undifferentiated stomach adenocarcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, and other seven types of cancers. Intriguingly, the amplification alteration frequency was up to 7% in undifferentiated STAD (Figure 4A). In adrenocortical carcinoma cases, the "deep deletion" alteration of CCL5 emerged uniquely with a frequency of about 3.5%. Mutations are a common form of genetic alteration. In esophageal squamous cell carcinoma cases, "mutation" alteration occurred exclusively with a frequency of ~1%. A missense mutation can induce gene code disorder. It was testified that W80*/C alteration of CCL5 existed at the 80th amino acid coding locus, which could convert methionine (M) into none or cysteine (C), inducing its frameshift mutation. We uncovered this W80*/C alteration in an esophageal carcinoma sample and a stomach adenocarcinoma case in the TCGA database (Figure 4B). DNA methylation is an epigenetic modification governing gene expression and cellular activity, which take a vital role in tumor pathogenesis. Thus, we applied "MEXPRESS" to explore whether a correlation existed between CCL5 and DNA methylation in 33 types of tumors. As shown in Fig. S1, CCL5 gene expression was negatively associated with DNA methylation in THCA, PRAD, LUSC, and BRCA, respectively, which indicated that CCL5 was hypomethylated in these tumor samples (for instance, cg10315334 probe showed a negative correlation with

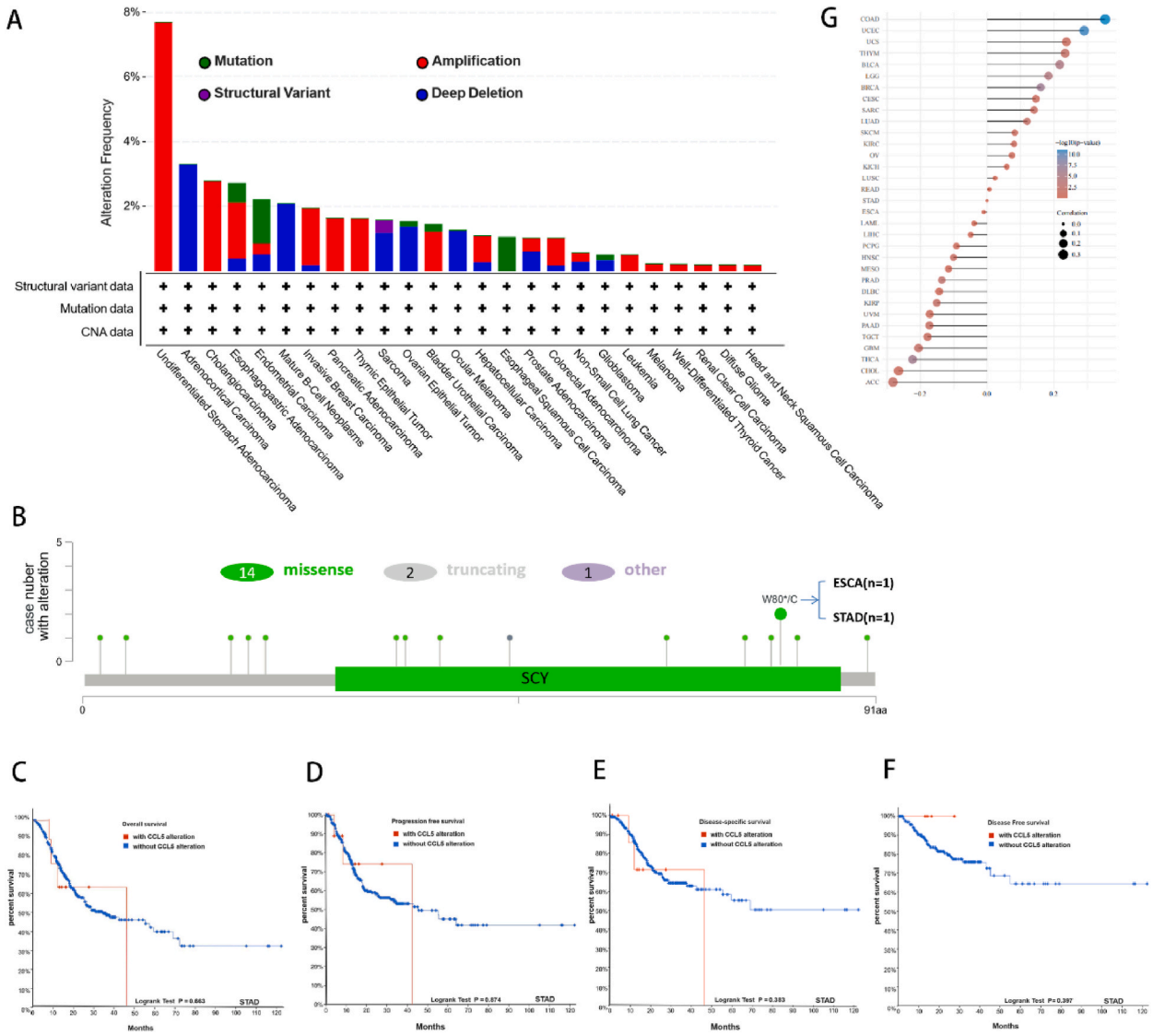

Figure 4. Genetic alteration of CLL5 existed in various tumors.

the Pearson R = 0.611 in BRCA).

Genetic alteration mediates tumor cells' biological activity, including differentiation, proliferation, and metastasis, which may affect clinical outcomes. Here, we observed that genetic alteration of CCL5 was significantly associated with clinical prognosis in stomach adenocarcinoma patients. As shown in Fig.4(C–F), overall survival, progression-free survival, disease-free survival, and disease-specific survival were worse in STAD patients with CCL5 alterations than those without alterations. Tumor mutational burden (TMB) is defined as the total number of nonsynonymous mutations, including base substitution, indel, and other mutations per coding area of the tumor genome. Recent studies testified that TMB was significantly associated with immunotherapeutic prognosis. Here, we

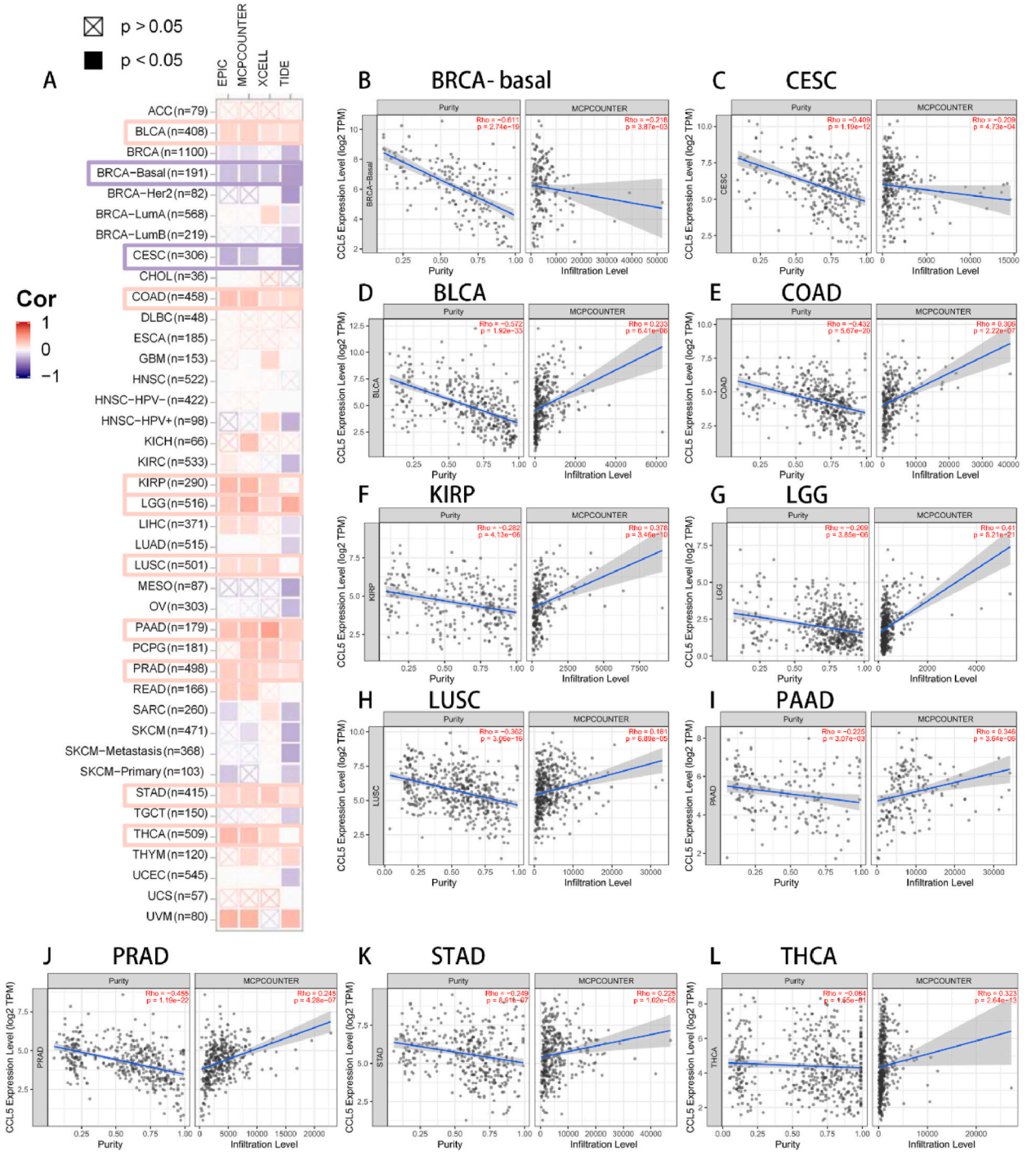

Figure 5. Correlation analysis between CCL5 expression and the immune infiltration level.

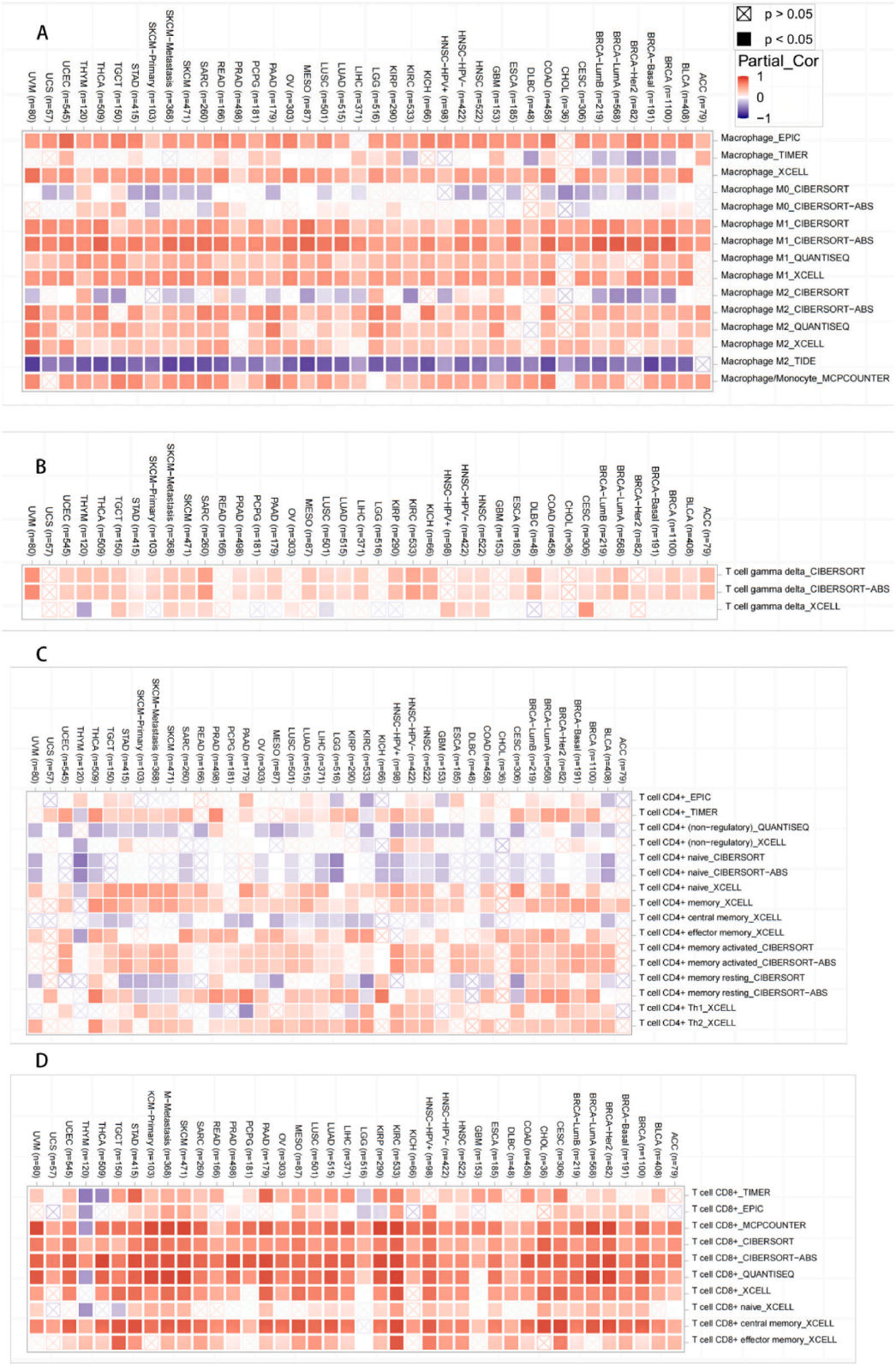

Figure 6. Correlation analysis between CCL5 expression and macrophage or T cell gamma delta.

investigated whether CCL5 is associated with TMB monitoring cancer clinical prognosis. As shown in Figure 4G, CCL5 was negatively associated with TMB in ACC, CHOL, THCA, and other 13 types of cancers; inversely, it was positively associated with TMB in 16 types of tumors, especially in COAD and UCEC cancer. It can be presumed from this that CCL5 might be a biomarker for prognosis, although many studies are needed to confirm it.

We analyzed the mutation features of CCL5 in 33 types of tumors using the cBioPortal tool. The alteration frequency with mutation type (A) and mutation site (B) was displayed. Next, we analyzed the potential correlation between mutation status and overall (C), disease-specific(D), disease-free (E), and progression-free survival (F) of STAD using the cBioPortal tool. Finally, the correlation between CCL5 expression and TMB from all types of tumors in TCGA was analysed (G).

The information of CCL5 DNA methylation was displayed in the table, respectively for LUSC (A), PRAD (B), BRCA (C), and THCA (D).

3.4. CCL5 expression was significantly associated with immune infiltration

The immune cells play vital roles in the tumor microenvironment, closely associated with tumor progression, metastasis, and prognosis. Numerous studies have investigated the immune infiltration of CD4+ T cells, macrophages, neutrophils, CD8+ T cells, macrophages, neutrophils, and other multiple immune cell subsets in the tumor microenvironment. We employed TIMER, CIBERSORT, quanTIseq, MCPCOUNTER, XCELL, xCell, and EPIC algorithms to explore the potential relationship between CCL5 expression and immune response cells infiltration in 33 types of tumors. All or almost algorithms showed that CCL5 was significantly associated with immune infiltration in 11 types of tumors (Figure 5A). From being calculated by the MCPCOUNT algorithm, data revealed that a

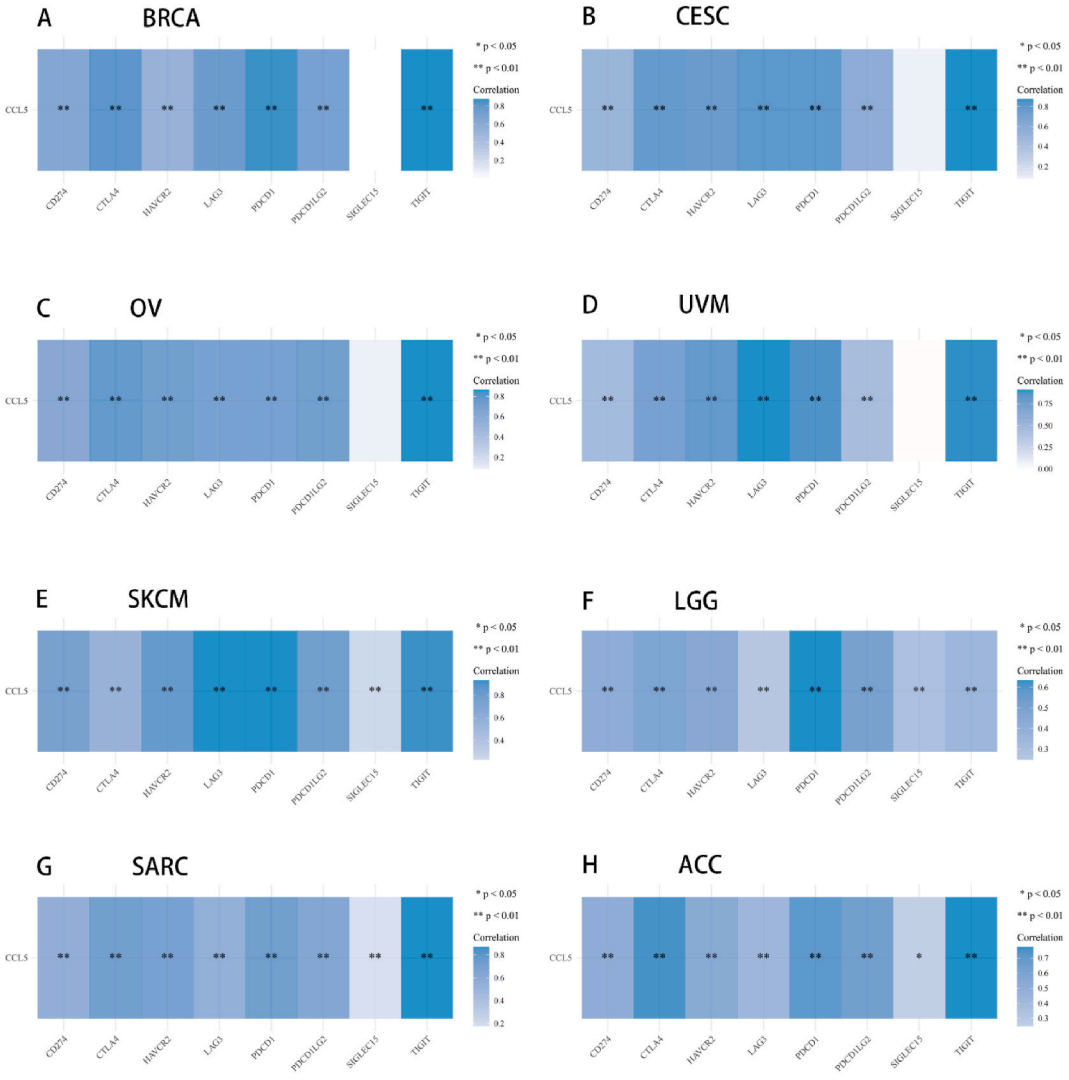

Figure 7. CCL5 expression was closely correlated with immune checkpoints.

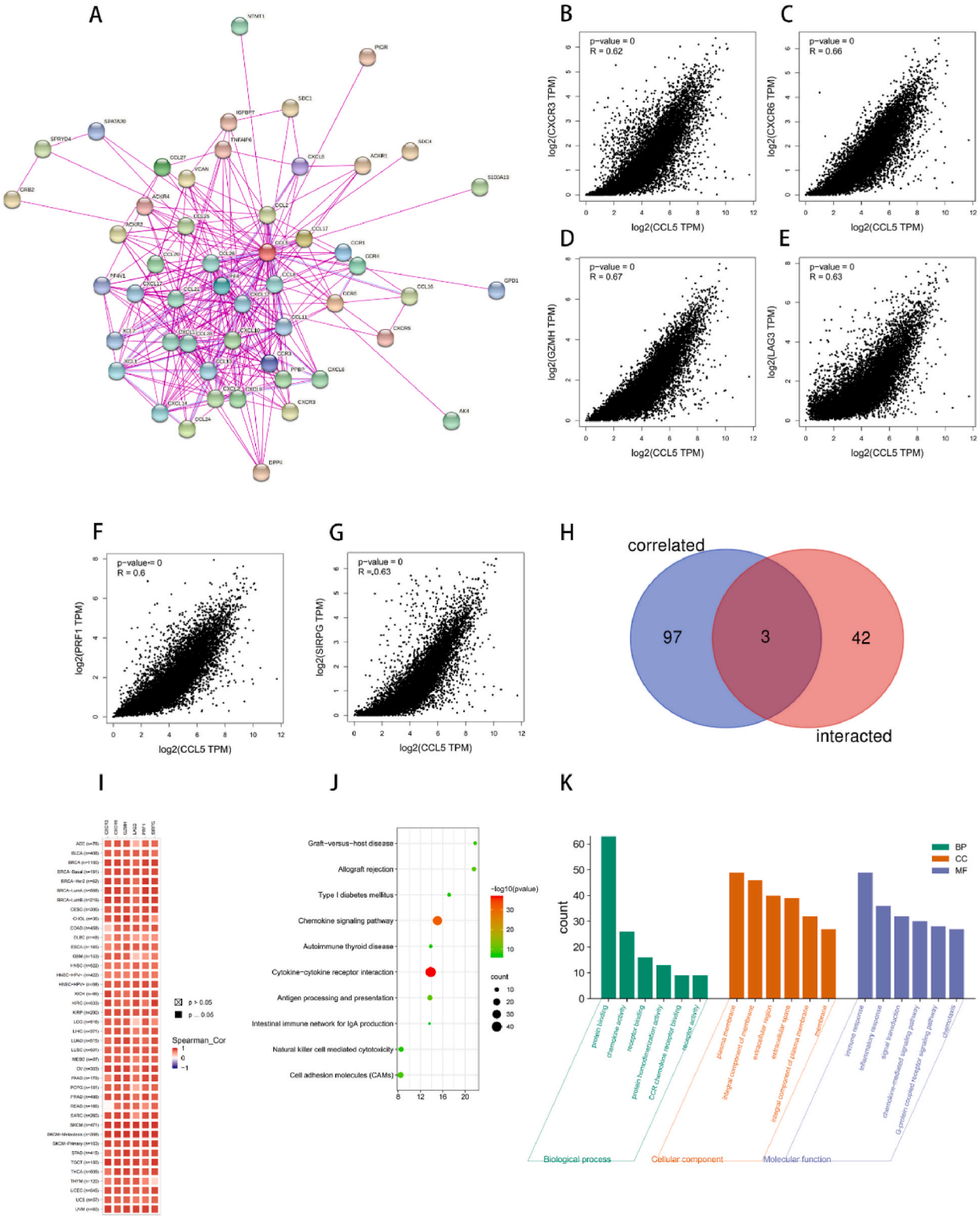

Figure 8. CCL5-related gene enrichment analysis.

negative correlation existed in BRCA-basal and CESC tumors (Fig.5B and Fig.5C); in contrast, a significantly positive correlation of that was observed in BLCA, COAD, STAD, KIRP, THCA, PRAD, LGG, PAAD, and LUSC in Fig.5 (D-L). Furthermore, we investigate the composition of immune cells infiltrating the tumor microenvironment. As shown in Fig.6A and Fig.6B, gamma delta T cells and macrophages had significantly positive correlations with CCL5 expression in most tumors. In addition, CCL5 was positively associated with CD4+ T cells infiltration in most types of tumors according to CIBERSORT and XCELL algorithms calculation (Figure 6C). Meanwhile, a significant positive correlation existed between CCL5 and CD8+ T cells infiltration in all the 33 types of tumors acquired from CIBERSORT algorithms (Figure 6D).

The potential correlation between CCL5 expression and the infiltration level of cancer-associated cells across all types of cancer in TCGA (A). The correlation between CCL5 expression and the infiltration level of cancer-associated cells for BRCA-basal, CESC, BLCA, COAD, KIRP, LGG, LUSC, PAAD, PRAD, STAD, and THCA, respectively shown from B to L.

The "Immune-Gene" module of the TIMER2 web server was used to explore the association between CCL5 expression and immune infiltrates from all types of tumors in TCGA. The EPIC, MCPCOUNTER, XCELL, QUANTISEQ, CIBERSORT-ABS, TIMER and CIBERSORT algorithms were utilized for immune infiltration estimations. We conducted the correlation analysis between CCL5 expression and macrophage (A), T cell gamma delta (B), CD4+ T cell (C) or CD8+ T cell (D).

3.5. CCL5 was associated with immune checkpoint molecules

Because immune infiltration level and immune checkpoint molecules were considered as monitors for evaluating tumor microenvironment and predicting the response of immunotherapy. After acquiring a close association between CCL5 expression and immune infiltration, we explored whether CCL5 is also associated with immune checkpoint molecules. Eight checkpoints were screened, including CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, SIGLEC15, and TIGIT. As shown in Figure 7(E-H), CCL5 expression was closely correlated with these eight checkpoint molecules in SKCM, LGG, SARC, and ACC patients ($p < 0.01$). Similarly, except for SIGLEC15, other seven checkpoint molecules were significantly associated with CCL5 expression in BRCA, CESC, OV, and UVM patients in Fig.7(A-D). These data indicated that CCL5 could be used as a new prognostic biomarker for immunotherapy.

TIDE (Tumor Immune Dysfunction and Exclusion) algorithm was usually employed to calculate the index score for predicting potential immunotherapy response. Studies manifested that the TIDE score was negatively associated with immunotherapy response and prognosis. Thus, we utilized it to investigate whether CCL5 affects the response of checkpoint inhibitors immunotherapy. Unfortunately, the number of LGG, SARC, and UVM patients who acquired immunotherapy was too small to analyze the response efficiency in the CIT database. Yet, no data was available for BRCA, OV, ACC, and CESC patients in the CIT database. However, we observed TIDE score was less in CCL5 high-expression group than that of the CCL5 low-expression group in SKCM patients ($p = 0.071$) (Fig. S2J), although the number of recruited patients was inadequate to achieve a significant statistic difference. It indicated that the CCL5 highly expressed subjects could acquire a better immune response to immunotherapy in SKCM patients. Moreover, we conducted the association analysis of the CCL5 express and the immunotherapy response in the CAMOIP web. Higher CCL5 express of HCC patients with immunotherapy is closed association with higher overall survival and free disease survival (Fig. S2E and Fig. S2F). Unfortunately, no association between CCL5 express with immunotherapy response in renal cell carcinoma, NSCLC, melanoma, BLCA and bladder cancer in Fig. S2(A-D) and Fig. S2(G-I).

1. The relationship between immune checkpoints and CCL5 expression in BRCA (A), CESC (B), OV (C), UVM (D), SKCM (E), LGG (F), SARC (G), and ACC (H). 2. *$P < 0.05$; **$P < 0.01$.

The relationship of CCL5 expression and Renal cell carcinoma (A), NSCLC (B and C), BLCA (D),Melanoma (E and F), HCC (G and H), and Bladder cancer (I). The TIDE score of SKCM patients was displayed (J). TIDE: Tumor Immune Dysfunction and Exclusion. high level: the high expression of CCL5. low level: the low expression of CCL5.

3.6. Enrichment analysis of CCL5-related partners

To further investigate the molecular mechanism of CCL5 in oncogenesis, we screened CCL5 binding-targeted proteins and CCL5 expression-correlated genes via pathway enrichment analysis. We obtained 45 binding-targeted proteins, which were testified by experimental data, and the interaction network of these targeted proteins showed in Figure 8A. Meanwhile, we identified the top 100 genes which significantly correlated with CCL5 expression. Six of them were selected as a sample to be displayed with closer correlation to CCL5 and well scatter plot, including CXC chemokine receptor 3 (CXCR3), CXC chemokine receptor 6 (CXCR6), Granzyme H (GZMH), lymphocyte-activation gene 3 (LAG3), Perforin-1(PRF1), and the signal regulatory protein gamma (SIRPG) in Fig.8(B-G). The heatmap also exhibited a positive association between CCL5 and these six genes mentioned above conformably in most types of cancer from the TCGA database (Figure 8I). From intersection analysis, we acquired CXCL9, XCL2, and CXCR3, which are overlapped members in CCL5 binding-targeted and CCL5 expression-correlated gene (Figure 8H). Then, we conducted KEGG and GO analysis. Data showed that cytokine-cytokine receptor interaction and chemokine signaling might be involved in the role of CCL5 in regulating tumor pathogenesis and prognosis (Figure 8J); these screened genes participate in chemokine signaling, receptor binding, immune response, inflammatory response, and other activities (Figure 8K).

First, we obtained the available experimentally determined CCL5-binding proteins by using the STRING tool (A). Second, we obtained the top 100 CCL5-correlated genes in TCGA projects by using the GEPIA2 approach and analyzed the expression correlation between CCL5 and selected targeting genes, including CXCR3, CXCR6, GZMH, LAG3, PRF1, and SIRPG (from B to G). The corresponding heatmap data in the various cancer types are presented (I). We also performed an intersection analysis of the CCL5-binding and correlated gene (H). Based on the CCL5-binding and interacted genes, we conducted KEGG pathway analysis (J). The molecular

function data in GO analysis was also displayed (K).

4. Discussion

The tumor microenvironment is a complex ecosystem where host immune system and cancer cells interact constantly. Tumor cells could disrupt a typical setting of chemokines and inflammatory cytokines, inducing tumorigenesis. Numerous studies have demonstrated that chemokines play essential roles in tumor development, metastases and prognosis [21, 22, 23]. CCL5 is secreted by platelets, T lymphocytes, macrophages, synovial fibroblasts, tubular epithelium, and tumor cells [8, 24, 25]. Its activity is mediated by not only binding to CCR5 with a high affinity but also by binding to CCR1, CCR3, CCR4, CD44, and GPR75 [26-28]. Numerous studies have shown that CCL5 was expressed in various cancers, including acute lymphocytic leukemia [29], Hodgkin lymphoma [10], breast cancer [13, 30, 31], pancreatic cancer [32], gastric adenocarcinoma [33, 34], prostate cancer [35, 36], and colorectal carcinoma [37]. Therefore, it has been considered that CCL5 plays a vital role in mediating signal transduction in tumors, promoting tumor invasion and metastasis in many solid tumors. However, a large number of researches have proved that CCL5 has an anti-tumor function by inhibiting proliferation, development, and metastasis, in addition to regulating the tumor microenvironment via recruiting immune cells and inducing inflammation [38]. Thus, it is like a double-edged sword in tumor biology. Pan-cancer is a fine approach to displaying molecules' distinctive roles in various types of tumors. Up to now, no pan-cancer analysis has been achieved to elucidate the role of CCL5 in regulating various tumor biologic processes, but it is crucial for guiding tumor immunotherapy in the future. In this study, we found that CCL5 over-expressed in most types of cancers, except for PCPG and CHOL. Discrepant expression of CCL5 in different pathological stages was observed in COAD, HNSC, KIRC, STAD, THCA, and SKCM. However, the discrepancy of CCL5 expression was irregular in different stages of those cancers, perhaps owing to CCL5's bi-directional role. Further in-depth studies need to illustrate the divergence of CCL5 expression in different pathological stages linked to its role in tumor invasion, metastasis, and prognosis.

Compared to adjacent normal tissues, CCL5 protein in the primary tissues of clear-cell RCC was higher. In contrast, its level in the primary tissues of LUAD and breast cancer was significantly lower than in normal tissues, which was inconsistent with Soria's finding that the expression of CCL5 was notably low in epithelial cells of benign breast lumps and normal ducts [24]. However, our data from TCGA were consistent with those of former studies in most types of cancers. Large previous studies found that CCL5 expression was closely correlated with prognosis in a variety of tumors. Our study found that CCL5 expression level was closely correlated with overall survival in BRCA, CESC, LGG, SARC, SKCM, THYM, and UVM patients. Meanwhile, it was closely correlated with disease-free survival in ACC, GBM, OV, and SKCM patients. CCL5 has been suggested as a tumor biomarker for monitoring breast cancer (stage II) progression [12]. Some studies found that the level of CCL5 expression was higher in advanced stages of tumors (e.g., stages II and III). However, a study reported CCL5 expression was not significantly different among the different stages of breast cancer, particularly in triple-negative breast cancer [39], but it was low in benign lesions and healthy individuals [12]. We found that highly expressed CCL5 was associated to well clinical outcome and disease-free survival in breast cancer patients, which was consistent with Fujimoto's report [40]. However, other studies have shown that CCL5 plays a significant role in the breast tumor progression and metastasis through β-catenin induction, whereby synergically up-regulated IL-6 and CCL5, or immune response [41, 42]. A quantitative analysis revealed that poor clinical outcomes were associated with CCL5 high expression in breast cancers, resulting from the serum level of CCL5 being higher in advanced stages. Therefore, it is somehow puzzling of the inconsistent results. In addition, the detailed mechanisms of CCL5 in favorable prognosis were also unclear. Thus, these issues needed to be elucidated in further studies with a large number of patients.

Due to immune checkpoints and their inhibitors being screened successfully, checkpoint inhibitor immunotherapy has become an advanced treatment for patients. In the past decades, we have witnessed CIT's rapid developments. However, only a small portion of patients benefited from the CIT, owing to a lack of enough immune response for immune checkpoint molecules, such as CD274 (also called PD-L1, programmed cell death protein 1), CTLA4 (cytotoxic T lymphocyte-associated protein 4), LAG3 (lymphocyte activation gene-3), etc [43, 44, 45]. The expression level of immune checkpoint such as CD274 and PDCD1 (also named PD-1) were potential biomarkers for predicting CIT response [44]. Several inhibitors targeting alternative immune checkpoints are in preclinical and clinical stages of development, including those targeting LAG3, HAVCR2 (TIM3), PDCD1LG2 (PD-L2), SIGLEC15, and TIGIT. The eight immune checkpiont molecules primarily change different pathways,resulting in inactivation of tumor-specific T cells and immune evasion [46]. CIT therapy can reinvigorate T cells and allows the adaptive immune system to target tumor cells. To understand the role of CCL5 in CIT, we investigated the correlation between the expression of checkpoint molecules and CCL5. Intriguingly, we found that CCL5 was significantly associated with the expression level of checkpoints, including in CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, SIGLEC15, and TIGIT eight types of tumors. We presume that CCL5 might be a biomarker for immunotherapy response in cancer patients.

Although studies indicated that increased expression of multiple chemokines and cytokines was closely associated with poor prognosis and metastasis [38, 47]. Chemokines derived from stroma cells, fibrosis cells, and even immune cells in the tumor microenvironment could recruit T cells, macrophages, NK, and other immune cells to scout tumor cells. In this study, we demonstrated a positive correlation between CCL5 expression and immune infiltration of CD4+T, CD8+ T cells, and macrophages in 33 types of tumor types. Therefore, uncovering chemokine-mediated biological processes and interfering in the tumor microenvironment are beneficial options for clinical treatment [48, 49]. In recent years, the tumor immune dysfunction and exclusion (TIDE) algorithm has been a new fine index to predict immunotherapy response in cancer patients [50]. A higher TIDE score means the response rate to immunotherapy is lower. Our data found that CCL5 high-expressed group acquired a low score in SKCM patients, while the CCL5 low-expressed group acquired a high score in SKCM patients. Our former data indicated that a low expression level of CCL5 acquired poor OS and DFS in SKCM patients. Thus, we inferred from the data displayed that CCL5 might contributes to the immune response of CIT in SKCM

patients. More research is needed to support our speculation.

This study has limitations. Firstly, we only used TCGA data and GTEx data for analysis, without experimental verification (Actually, we are conducting experimental verification). Secondly, during the data analysis process, we used different tools and algorithms. Results may vary due to differences in data inclusion and processing. Thirdly, when analyzing the role of CCL5 in immunotherapy, the results could not be counted due to the small number of cases of some tumors. More research is needed. Despite its shortcomings, we cannot deny the significance of the pan-cancer analysis of CCL5. It indicates the direction for the following research.

5. Conclusion

In conclusion, as we known, our study is the first pan-cancer analysis focusing on the role of CCL5 in affecting on clinical prognosis, immune infiltration, and response to CIT in a variety of cancers. Notably, data indicated that CCL5 might be used as an independent prognostic monitor for various cancer patients, especially for SKCM patients, which is conducive to the precise treatment of cancer. However, further in-depth experimental studies need to verify our conclusion in the future.

Author contribution statement

Yanchun Huang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Lijuan Wu: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Yong Sun and Jiwen LI: Performed the experiments; Analyzed and interpreted the data.
Nan Mao and Yeqing Yang: Contributed reagents, materials, analysis tools or data.
Ming Zhao and Sichong Ren: Conceived and designed the experiments.

6. Data availability statement

No data was used for the research described in the article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18215.

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