Comprehensive Characterization And Clinical Relevance of CLEC4G Expression Across Human Cancers

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Abstract

Liver and lymph node sinusoidal endothelial cell C-type lectin (CLEC4G) interacts with the surface glycoproteins of enveloped viruses and mediates immune evasion for viruses. Recent studies have indicated that the expression of CLEC4G affects the development and microenvironment of tumors. In the present study, we revealed comprehensive characterization of CLEC4G expression across human cancers. We first explored the effect of CLEC4G expression on overall survival (OS) across human cancers. High expression of CLEC4G was beneficial in lung adenocarcinoma (LUAD) and detrimental in kidney renal clear cell carcinoma (KIRC) and uveal melanoma (UVM) in terms of OS. Enrichment analysis of the 14 cancer-related signatures suggested that CLEC4G regulated tumor metastasis and immune response. Enrichment analysis of the immunophenotypes and cancer-immunity cycles indicated that CLEC4G was involved in regulation of macrophages and Treg cells, which affected tumor immune response. We also observed correlations between CLEC4G expression and checkpoint molecular expression in different cancer types. Taken together, the present study revealed the comprehensive characterization of CLEC4G expression across human cancers, predicting the roles of CLEC4G in the development and immune microenvironment of tumors.

Introduction

Liver and lymph node sinusoidal endothelial cell C-type lectin (CLEC4G, also named LSECtin), a Ca^{2+} -dependent C-type lectin, is a type II transmembrane glycoprotein known to interact with mannose, N-acetylglucosamine (NAcGlc), and fucose. CLEC4G has been detected in the liver [1], lymph nodes [2], myeloid cells [3], macrophages [4] and melanoma cells [5]. Previous studies indicated that CLEC4G interacted with the surface glycoproteins of enveloped viruses and mediated immune evasion for viruses, such as encephalitis, SARS and Ebola virus [6–10].

The roles of CLEC4G in cancer growth and progression has become a focus of attention in recent years. Enhanced expression of CLEC4G increases colon cancer adhesion, migration and liver metastasis [11]. CLEC4G on tumor-associated macrophages promotes breast cancer stemness and growth through the CLEC4G/butyrophilin 3A3 (BTN3A3) signaling axis [4]. Interestingly, CLEC4G expression suppresses antitumor T-cell responses in melanoma cells, leading to tumor progression [5, 12]. CLEC4G inhibits human T cell activation and proliferation and decreases cytokines (including IFN- γ , IL-2, IL-17 and TNF- α) and chemokines (including IL-8/CXCL8, MIG/CXCL9, IP-10/CXCL10, and TARC/CCL17) generations [12]. These findings suggest important roles of CLEC4G in cancer progression mediated by regulating immune cells.

Next-generation sequencing (NGS) technology and bioinformatics analysis have been widely applied in high-throughput analysis and molecular diagnosis studies [13–16]. These technologies and methods also enable researchers to quickly explore and reveal gene functions and effects in a pan-cancer cohort. In the present study, we screened different cancer types and found a significant correlation between CLEC4G expression and overall survival (OS). We further performed enrichment analysis in the 14

manually curated cancer-related signatures, immunophenotypes and cancer-immunity cycles for CLEC4G expression in cancers. In addition, the relationship between CLEC4G expression and checkpoint molecular expression was explored.

Materials And Methods

Data collection and processing

A total of 9640 patients with a pan-cancer cohort were obtained from the Cancer Genome Atlas (TCGA) database, whose gene expression profiles, copy number data and clinical data are available in TCGA (<https://gdc.cancer.gov/about-data/publications/panimmune>) [17].

To evaluate the functional states of cancer cells, we calculated 14 manually curated cancer-related signatures [18] (stemness, invasion, metastasis, proliferation, EMT, angiogenesis, apoptosis, cell cycle, differentiation, DNA damage, DNA repair, hypoxia, inflammation and quiescence) using the GSVA package in R. To quantify the proportions of immune cells in the tumor microenvironment, we applied single-sample gene set enrichment analysis (ssGSEA) [19], allowing for the assessment of 28 immune cell types. To reveal the interaction of immune cells and cancer cells in the tumor microenvironment, we performed cancer-immunity cycle enrichment analysis [20], including GS1, GS2, GS3, GS4, GS5, GS6, GS7 and GS8. GS1 represented existence of T-cell immunity; GS2 represented tumor antigenicity; GS3 represented priming and activation; GS4 represented trafficking and infiltration; GS5 represented recognition of tumor antigens; GS6 represented absence of inhibitory cells; GS7 represented absence of checkpoint expression; and GS8 represented absence of inhibitory molecules.

Statistical analysis

Statistical analyses were conducted using R software (version 3.4.2.). Data were expressed as the median and interquartile range (IQR). Two group values were analyzed for differences by the Wilcoxon rank-sum test. The Kruskal-Wallis test was used for more than two groups to test differences. A log-rank test and Kaplan-Meier curves were used to compare OS. $p < 0.05$ was considered significant with two-sided tests.

Results

CLEC4G expression in different cancer types

We first explored the expression of CLEC4G in tumor samples and paired normal tissues from different cancer types using the Gene Expression Profiling Interactive Analysis (GEPIA). Transcripts per million (TPM) data of CLEC4G indicated that CLEC4G expression was detected in all tumor types (Table S1) and four tumors (including BRCA, GBM, LGG and LIHC) exhibited significant differences between tumor samples and paired normal tissues (Figure S1A). The TPM values of CLEC4G were significantly

decreased in BRCA, GBM, LGG and LIHC compared with paired normal tissues, suggesting that the expression of CLEC4G in the four tumors was reduced (Figures S1A and S1B).

Effect of CLEC4G expression on OS in different cancer types

To investigate the effect of CLEC4G expression on OS, the TPM value of CLEC4G as a continuous variable was analyzed with OS using univariate Cox hazard analysis in different cancer types. As shown in Figure 1A, higher expression of CLEC4G in GBM, KIRC, STAD and UVM was associated with a worse OS. The HRs and their 95% CIs for OS were 1.2 (1-1.3), 1.4 (1.2-1.5), 1.1 (1-1.3) and 1.9 (1.3-2.8) in GBM, KIRC, STAD and UVM, respectively. However, higher expression of CLEC4G in LGG was associated with a better OS (HR 0.87, 95% CI (0.77-0.97), $p = 0.017$) (Figure 1A).

Further, 75%, 50% and 25% of CLEC4G expression were used as cutoff points and we divided the cancers into high expression and low expression groups. We explored the correlation of the high/low groups with OS in different cancer types. Log-rank test results indicated that high expression of CLEC4G showed a longer OS in LUAD patients when 50% or 25% was considered as the cutoff point (Figure 1B). However, high expression of CLEC4G exhibited a shorter OS in KIRC patients when 75% or 50% of CLEC4G expression was considered as the cutoff point and in UVM patients when 75%, 50% or 25% was considered as the cutoff point (Figure 1B). Then we presented the association of high/low expression of CLEC4G and OS using cutoff points of 50% or 25% CLEC4G expression in LUAD, KIRC and UVM (Figure 2B and 2C). The results were consistent with Figure 1B.

We further explored effects of expression of CLEC4G on survival analysis in different pathologic stages of LUAD, KIRC and UVM. Clinical characteristics of high/low expression of CLEC4G in the LUAD, KIRC and UVM cohorts were exhibited in Table S2-4, respectively. The results indicated that high expression of CLEC4G was beneficial in stage II LUAD patients except for stage I, III and IV (Figure S2). In the KIRC cohort, high expression of CLEC4G in stage II, III and IV KIRC was detrimental except for stage I (Figure S3). In the UVM cohort, there was no significant change in the stage III UVM cohort, but the high expression group was beneficial for OS in the stage II UVM cohort (Figure S4). Because of no stage I patient and few stage IV patients, survival analysis was not performed in UVM. These data indicated that effects of CLEC4G were different on survival in different cancers and stages. High expression of CLEC4G was beneficial in LUAD and detrimental in KIRC and UVM in terms of OS. CLEC4G expression had a weak role in stage I LUAD and KIRC, which suggested that CLEC4G involved in tumor metastasis.

Correlation Analysis Of Clec4g Expression And 14 Cancer-related Signatures

We further explored the enrichment of CLEC4G expression in 14 manually curated cancer-related signatures (including stemness, invasion, metastasis, proliferation, EMT, angiogenesis, apoptosis, cell cycle, differentiation, DNA damage, DNA repair, hypoxia, inflammation and quiescence). In the pan-cancer analysis, the expression of CLEC4G was significantly negatively correlated with inflammation,

differentiation, angiogenesis, quiescence, EMT, invasion, metastasis, proliferation, apoptosis, hypoxia and stemness signatures and significantly positively correlated with DNA repair and DNA damage signatures (Figure 3A). Signatures about the occurrence and development of cancer and inflammation signature were significantly enriched in KIRC, UVM and LUAD with high expression of CLEC4G (Figures 3B-D). DNA repair and damage signatures were enriched in KIRC, UVM and LUAD with low CLEC4G expression (Figure 3B-D). These data indicated that CLEC4G expression regulated cancer development and inflammation generation in the pan-cancer cohort, KIRC, UVM and LUAD.

Effects of CLEC4G expression on immune cells and cancer cells

Enrichment analysis of cancer-related signatures revealed that CLEC4G expression regulated the inflammation signature. To explore effects of CLEC4G on immune response, we characterized the immunophenotypes for high/low expression of CLEC4G and visualized the enrichment of immune subpopulations. The result showed that TILs were associated with the expression of CLEC4G in the pan-cancer analysis (Figure 4A). CD56bright NK cell, effector memory CD4 T cell, and activated DC cell, CD4 T cell and CD8 T cell were not significantly enriched in KIRC, but CD56dim NK cell was enriched in KIRC with high expression of CLEC4G (Figure 4B). Monocytes, immature DC cell, neutrophil, Th2 cell, Th17 cell and memory B cell were significantly enriched in UVM (Figure 4C). In LUAD, except for CD56dim NK cell, the other immune cell types were significantly enriched in patients with high CLEC4G expression (Figure 4D). Meanwhile, we found that CLEC4G expression was significantly negatively correlated with macrophage and regulatory T cell in the pan-cancer cohort, KIRC and LUAD (Figure 4A, B and D), suggesting that CLEC4G could regulate functions of macrophages and regulatory T cells.

We further investigated an immunogram for the cancer-immunity cycle to evaluate the interaction of immune cells and cancer cells in the tumor microenvironment [20]. In the pan-cancer analysis, the expression of CLEC4G was associated with 8 immunogram scores (Figure 4E). The correlation was different in different cancer types (Figures 4F-H). GS6 and GS8 were markedly enriched in KIRC with high expression of CLEC4G, suggesting a T-cell-poor phenotype (Figure 4F). GS5 and GS7 were markedly enriched in UVM with high expression of CLEC4G, suggesting a T-cell-intermediate phenotype (Figure 4G). In LUAD with high CLEC4G expression, GS1 was significantly enriched, suggesting a T-cell-rich phenotype (Figure 4H). These data showed that high expression of CLEC4G regulated the GS6 process, which was presented as the absence of inhibitory cells (Treg and MDSC enrichment) [20]. These results were consistent with the immunophenotypes analysis. Thus, CLEC4G expression might regulate immune cells (macrophages, Treg et. al) to impact cancer development.

Correlation Analysis Between Clec4g Expression And Checkpoints

The expression of CLEC4G and checkpoints was further evaluated in different cancers. We found that the expression of CLEC4G was weakly correlated with the expression of 47 checkpoints in many cancer

types, such as DLBC, GBM, KIRP and LIHC (Figure 5A). In UVM, the expression of CLEC4G was significantly positively correlated with most checkpoints (Figure 5A). These data might provide a guide for studies correlating CLEC4G and checkpoint expression in different cancers.

Discussion

CLEC4G, as one of DC-SIGN family members, is found in many tissues and cells and our data also revealed that CLEC4G expression was detected in many tumor cells and paired normal tissues, although the expression level was different between these tissues. Survival analysis surprisingly indicated that CLEC4G expression significantly affected OS in stage II LUAD, KIRC and UVM, although survival outcome was opposite between LUAD, and KIRC and UVM. Lymph node metastasis is considered an important symbol of stage II in pathologic stages of cancers [21–23]. Thus, CLEC4G might regulate tumor cell metastasis and promote tumor development. Recent studies indicate that CLEC4G expression is involved in the growth and progression of tumors [4, 11]. CLEC4G expression promotes tumor progression in melanoma cells in mouse models [5] and colon carcinoma cell adhesion, migration and *in vivo* metastasis to the liver in colon cancer patients [11]. However, high expression of CLEC4G, interestingly, showed a longer OS in LUAD (n = 506) in the present study from the TCGA dataset. In contrast, a study showed that LUAD patients with positive CLEC4G expression had a significantly lower survival rate [24]. We were unable to explain the reason according the current data. The further demonstration is needed about effects of CLEC4G on survival in the future study.

CLEC4G participates in cell adhesion and pathogen recognition roles [25, 26]. Function enrichment of CLEC4G expression showed that CLEC4G expression was correlation with enrichment of differentiation, angiogenesis, quiescence, EMT, invasion, metastasis, stemness and proliferation signatures in the pan-cancer cohort. Previous research found that CLEC4G increased hepatic metastases of LS174T and LoVo cells and the results were demonstrated in a CLEC4G-knockout mouse model and a colon cancer patient cohort [11]. The study, meanwhile, revealed that CLEC4G promoted c-Met expression in colon cancer cells [11]. *Met* is considered as a metastasis and stemness-related gene across all cancer types [18]. We also found that metastasis and stemness signatures were enriched in pan-cancer, KIRC and LUAD cohorts. Since the demonstration of the function and roles of CLEC4G is still insufficient in tumor metastases, further research is needed in different cancers.

The inflammation signaling signature was enriched in the pan-cancer cohort and KIRC, UVM, and LUAD with high CLEC4G expression, which suggested that CLEC4G is related to the immune response in the tumor microenvironment. Immunophenotype analysis revealed that macrophages and T cells were significantly enriched in different cancers with high expression of CLEC4G. CLEC4G on tumor-associated macrophages interacts with BTN3A3 on breast cancer cells and promotes breast cancer stemness and growth [4]. CLEC4G is involved in macrophage-mediated intestinal repair in inflammatory bowel disease [27, 28]. In addition, it has been demonstrated that the T-cell immune response is negatively regulated by CLEC4G expression [29]. CLEC4G expression suppresses antitumor T-cell responses through interaction with the butyrophilin family receptor BTN3A1 in T cells [5, 12, 30]. Meanwhile, an immunogram of the

cancer-immunity cycle showed that CLEC4G expression impacted GS6 (the absence of inhibitory cells (Treg and MDSC enrichment)), which was consistent with results of immunophenotype analysis. Thus, macrophages and Treg cells might be considered as the further research direction to the tumor immune microenvironment in cancers.

Taken together, the present study provided many potential correlations between CLEC4G expression and OS, cancer-related signatures, immunophenotypes, cancer-immunity cycles and checkpoint molecules in pan-cancer and different type cancer cohorts. Although the current studies about roles of CLEC4G in different cancers are less, we hope that our research provides a valid directive for subsequent exploration.

Conclusion

Our research reveals the correlations of CLEC4G expression with OS in different cancers. CLEC4G expression is beneficial to OS in LUAD and detrimental to OS in KIRC and UVM. Enrichment analysis of the cancer-related signatures suggests that CLEC4G regulates tumor metastasis and immune response. Further analysis indicates that macrophages and Treg cells may be regulated by CLEC4G, which affects tumor immune response. Moreover, correlations between CLEC4G expression and checkpoint molecules are exhibited. The present study reveals the comprehensive characterization of CLEC4G expression across human cancers.

Declarations

Ethics approval and consent to participate

All data were downloaded from the TCGA (TCGA, PanCancer Atlas) via the cBioPortal (<http://www.cbioportal.org/>). TCGA belong to public databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

Consent for publication

All authors declare that there is no conflict of interest.

Availability of data and materials

The original data in this study can be found in the TCGA database (<https://gdc.cancer.gov/about-data/publications/panimmune>). The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interest.

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Authors' contributions

YL, FW, and LG conceived and designed the study; YL, HH, XY, XL, ZH, LG and FW analyzed and interpreted the data; YL and LG wrote the manuscript; FW reviewed the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Effect of CLEC4G expression on OS in different cancer types. (A) Forest plots of TPM value of CLEC4G on OS. (B) Kaplan-Meier analysis of OS comparison between high and low expression of CLEC4G in different cancer types. 75%, 50% and 25% of CLEC4G expression, as cutoff points respectively, divided into a high group and a low group. The round black frame represented $p < 0.05$.

Figure 2

Survival analysis in LUAD, KIRC and UVM. (A-C) Kaplan-Meier curves of OS comparison between high and low expression of CLEC4G in LUAD (A), KIRC (B) and UVM (C) when 50% or 25% of CLEC4G expression was considered as the cutoff point. The Kaplan-Meier analysis and curve with log-rank tests were used to determine the significance of differences between the high and low expression groups.

Figure 3

Correlation of CLEC4G expression and 14 cancer-related signatures. (A-D) Enrichment of CLEC4G expression in 14 manually curated cancer-related signatures in the pan-cancer cohort (A), KIRC (B), UVM (C) and LUAD (D). The median CLEC4G expression, as a cutoff point, was divided into high and low groups. Blue represented high/low < 1; red represented high/low > 1.

Figure 4

Effects of CLEC4G expression on immune cells and cancer cells. (A-D) Enrichment of CLEC4G expression in the immune subpopulations in the pan-cancer cohort (A), KIRC (B), UVM (C) and LUAD (D). The median CLEC4G expression, as a cutoff point, was divided into high and low groups. Blue represented high/low < 1; red represented high/low > 1. (E-H) Enrichment of CLEC4G expression in the cancer-immunity cycle in the pan-cancer cohort (E), KIRC (F), UVM (G) and LUAD (H). The median CLEC4G expression, as a cutoff

point, was divided into high and low groups. Blue represented high/low < 1; red represented high/low > 1. GS1, existence of T-cell immunity in the tumor; GS2, tumor antigenicity; GS3, priming and activation; GS4, trafficking and infiltration; GS5, recognition of tumor antigens; GS6, absence of inhibitory cells; GS7, absence of checkpoint expression; GS8, absence of inhibitory molecules.

Figure 5

Correlation analysis between CLEC4G expression and checkpoints. Heat map of the correlation between CLEC4G and checkpoints in different cancers.

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