Immunological and clinicopathological characteristics of C1RL in 2120 glioma patients

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Abstract

Background: Glioma is a deadly and immunosuppressive brain tumour. Complement C1r subcomponent like (C1RL), a prognostic biomarker in several kinds of tumours, has attracted increasing attention from oncologists. However, the role of C1RL in glioma remains unclear.

Methods: Through analysis of 2120 glioma patients from 5 public datasets, the relationships between C1RL expression and clinicopathological characteristics were evaluated. Furthermore, the C1RL-associated genes were screened, and Gene Ontology (GO) analysis was conducted to investigate biological process enrichment. In addition, tumour purity, leukocyte infiltration and overall survival were evaluated based on C1RL expression.

Results: We found that C1RL expression was upregulated in glioblastoma (GBM), especially mesenchymal GBM and primary GBM. Increased C1RL expression accompanied the IDH1-wt phenotype in both lower grade glioma (LGG) and GBM. C1RL-associated genes were mainly enriched in biological processes related to the immune response. C1RL expression was also correlated with reduced tumour purity and increased M2 macrophage infiltration. Higher C1RL expression predicted unfavourable survival in patients with glioma and therapeutic resistance in GBM.

Conclusions: Our results imply that C1RL is involved in immunological activities and is an independent unfavourable prognostic biomarker in patients with glioma. C1RL is a potential clinical immunotherapeutic target for glioma treatment in the future.

Keywords: Glioma, C1RL, Immunosuppression, Unfavourable survival, Therapeutic resistance

Background

Glioblastoma (GBM; WHO grade IV) and lower grade glioma (LGG; WHO grade II and III) are incurable brain tumour. Existing therapeutic strategies only prolong the survival of glioma patients to a limited extent. Patients with glioma eventually die from tumour recurrence, even with aggressive treatment. Novel therapies that have been successful in other tumours, such as PD-1 inhibition [1] and bevacizumab administration [2, 3], have failed to extend the overall survival time of patients with glioma. Tumour treating fields (TTF), a novel therapy that was recently approved for GBM treatment by the Food and Drug Administration (FDA), is not widely used in clinical practice because of its high price and difficult process [4, 5]. The current poor situation pushes us to explore the mechanism of glioma development and identify novel therapies.

The immunosuppressive microenvironment significantly contributes to the progression and therapeutic resistance of glioma. On the one hand, glioma cells induce a relatively weak immune response and enhance immunosuppression. Compared to other malignancies, glioma exhibits a lower
mutational burden and fewer infiltrating T cells [6]. GBM cells block T cell activation and proliferation in response to T cell receptor stimulation by generating extracellular vesicles carrying PD-L1 [7]. Glioma cells promote the expression of PD-L1 on macrophages derived from healthy donors [8, 9]. Intratumoral immunosuppressive education by glioma also contributes to the rise of systemic immunosuppressive myeloid-derived suppressor cells (MDSCs) [10]. On the other hand, the brain provides an immunosuppressive environment for glioma. Compared to melanoma in the flank, melanoma in the brain contains fewer CD8 T cells [11]. Moreover, antigen-specific cytotoxicity is systemically impaired in mice with brain melanoma [11]. Naïve T cells are sequestered in large numbers in the bone marrow in cancer patients. This phenomenon characterizes a variety of tumours only when the tumours are located in the intracranial compartment [12].

Complement C1r subcomponent like (C1RL) was found to be a prognostic marker in hepatocellular carcinoma [13] and renal cell cancer [14]. A gene-based analysis showed significant associations between non-Hodgkin lymphoma or diffuse large B-cell lymphoma and the C1RL gene [15]. C1RL also mediates the progression of Burkitt's lymphoma [16]. C1RL is a protein-coding gene associated with ovarian adenocarcinoma and leucorrhea. In terms of molecular function, the C1RL protein, which is homologous to C1r, is identified as the active form of serine hydrolase [17]. The C1RL protein cleaves prohaptoglobin in the endoplasmic reticulum [18]. In addition, pro-C1s is proteolytically cleaved into two fragments with sizes identical to those of the two chains of active C1s by the C1RL protein [19]. However, the immunological and clinicopathological characteristics of C1RL in glioma remain unclear.

In the present study, we employed 2120 glioma specimens and 23 non-tumour brain tissues from 5 datasets to explore the clinicopathological and biological characteristics of C1RL in glioma. The clinicopathological features evaluated included WHO grade, histology, GBM status, IDH mutation status, GBM subtype, overall survival and therapeutic resistance. The biological process enrichment of C1RL-associated genes was analysed to explore the biological characteristics of C1RL. Moreover, the relationships between C1RL expression and tumour purity or leukocyte infiltration were analysed.

**Methods data collection**

Five datasets including transcriptomic files and corresponding clinicopathological information for patients who were diagnosed with glioma (WHO II-IV) were downloaded. A microarray dataset containing 539 samples (TCGAmic) and an RNA sequencing dataset containing 702 samples (TCGAseq) were downloaded from The Cancer Genome Atlas (TCGA; https://xenabrowser.net). A microarray dataset containing 301 samples (CGGAmic) and an RNA-sequencing dataset containing 325 samples (CGGAseq) were downloaded from The Chinese Glioma Genome Atlas (CGGA; http://www.cgga.org.cn/). A microarray dataset containing 276 samples (GSE16011mic) was downloaded from Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/).

**Statistics of C1RL expression patterns**

An unpaired t test was used in comparisons of C1RL expression between two groups. Ordinary one-way ANOVA (multiple comparisons) was applied to compare C1RL expression among three or more groups. P < 0.05 was considered significant.

**C1RL-associated gene sifts and gene ontology (GO) analyses**

Pearson correlation coefficients between C1RL and all other genes were calculated in RStudio 1.1.453 with the cor.test algorithm. C1RL-associated genes were defined as genes with an r value > 0.4 in the GBM dataset (TCGA-mic) and r > 0.5 in the glioma datasets (TCGAseq, CGGA-mic, and CGGAseq). All the C1RL-associated genes were introduced into DAVID (https://david.ncifcrf.gov/) for further GO analyses. The top 10 biological process terms of the GO analysis results are listed in Fig. 2.

Moreover, the detailed correlations between C1RL and immunosuppressive genes (CD86, LGALS9, and TGFB1) are shown in Fig. 3.

**Tumour purity and leukocyte infiltration**

The ESTIMATE algorithm package was used to analyse tumour purity. The CIBERSORT tool (https://cibersort.stanford.edu/) was used to evaluate leukocyte infiltration. Heatmaps were produced in MORPHEUS (https://software.broadinstitute.org/morpheus/) online. The colour shows the Z score (subtract mean, divided by standard deviation) of all the expression data. The samples were ordered according to the expression of C1RL.

**Survival analyses**

The log-rank test and Kaplan-Meier survival curves were used to describe survival differences between two groups. The survival analysis of the GSE16011 dataset was conducted in R2 (https://hgserver1.amc.nl/cgi-bin/r2/main.cgi).

**Results**

C1RL expression was upregulated in GBM, especially mesenchymal GBM, primary GBM and IDH1-wt GBM

In this study, we employed 2120 glioma specimens and 23 non-tumour brain tissues from 5 datasets. The characteristics and clinical information of the 5 datasets were summarized in Table S1.
**C1RL** expression was analysed according to the WHO classification, GBM subtype, GBM status and **IDH1** mutation status. First, the expression of **C1RL** was always highest in GBM in the 5 datasets according to both the grading system and the histology system (Fig. 1A-H). However, the expression levels of **C1RL** in GBM samples varied greatly. Furthermore, **C1RL** expression among different subgroups of GBM was analysed. Among the four transcriptomic subgroups of GBM, **C1RL** expression was always highest in mesenchymal GBM (Fig. 1I-L). Secondary GBM was developed from lower grade glioma and exhibited lower **C1RL** expression than primary GBM (Fig. 1M-N). The **IDH** mutation status is a well-accepted marker for glioma classification. **C1RL** expression was higher in **IDH1**-wt GBM than in **IDH1**-mt GBM (Fig. 1O-Q). In addition, **C1RL** expression was higher in **IDH1**-wt LGG than in **IDH1**-mt LGG (Fig. 1P and Q). These results suggested that higher **C1RL** expression accompanies more advanced malignancy in glioma, especially in GBM.

| A | C1RL, Histology, TCGA mic, n=539 |
| --- | --- |
| B | C1RL, Grades, CGGA seq, n=325 |
| C | C1RL, Grades, CGGA mic, n=301 |
| D | C1RL, Grades, GSE16011 mic, n=276 |
| E | C1RL, Pathology, TCGA seq, n=702 |
| F | C1RL, Pathology, CGGA seq, n=325 |
| G | C1RL, Pathology, CGGA mic, n=301 |
| H | C1RL, Pathology, GSE16011 mic, n=276 |
| I | C1RL, Subtypes, TCGA mic, n=529 |
| J | C1RL, Subtypes, TCGA seq, n=152 |
| K | C1RL, Subtypes, CGGA seq, n=65 |
| L | C1RL, Subtypes, CGGA mic, n=109 |
| M | C1RL, GBM status, CGGA seq, n=115 |
| N | C1RL, GBM status, CGGA mic, n=119 |
| O | C1RL, IDH1, TCGA mic, n=230 |
| P | C1RL, IDH1, CGGA seq, n=325 |
| Q | C1RL, IDH1, CGGA mic, n=297 |

Fig. 1 Histopathological characteristics of **C1RL** in glioma. **A**, **C1RL** expression in primary GBM (p-GBM) and nontumour brain tissues in TCGA mic. **B**, **C1RL** expression in distinct WHO grades of glioma in CGGA seq, CGGA mic, and GSE16011 mic. **C**, **C1RL** expression in distinct histological types of glioma in TCGA seq, CGGA seq, CGGA mic, and GSE16011 mic. **D**, **C1RL** expression in four GBM subtypes in TCGA mic, TCGA seq, CGGA seq, and CGGA mic. **E**, **C1RL** expression in **IDH1**-mutant or **IDH1**-wild-type LGG or GBM in TCGA mic. **F**, **C1RL** expression in samples with different GBM statuses in CGGA seq and CGGA mic. **G**, **C1RL** expression in **IDH1**-mutant or **IDH1**-wild-type LGG or GBM in TCGA mic. **H**, **C1RL** expression in samples with different GBM statuses in CGGA seq and CGGA mic. **O**, oligodendroglioma; AO, anaplastic oligodendroglioma; OA, oligoastrocytoma; AOA, anaplastic oligoastrocytoma; A, astrocytoma; AA, anaplastic astrocytoma. Mut, mutant; wt, wild-type. *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001
**C1RL-associated genes were enriched in the biological processes of the immune response**

The biological function of C1RL, especially in tumours, has not been clarified thoroughly. Therefore, we aimed to identify the possible biological function of C1RL through analysis of the biological functions of C1RL-associated genes. C1RL-associated genes were defined as genes with expression trends similar to those of C1RL in glioma samples. All the C1RL genes from each dataset were listed in Table S2 and were evaluated by GO analysis. The biological processes are listed in reverse order of their p values. The GO analyses showed that C1RL-associated genes were mainly enriched in the biological processes of the immune response, inflammatory response, IFN-γ mediated signalling pathway, and innate immune response (Fig. 2.A-D).

To determine whether C1RL plays a positive role in the anti-glioma immune response, the expression relationships between C1RL and existing biomarkers were analysed. The CD86 protein is the receptor of CTLA4 and is mainly expressed on dendritic cells and monocytes. Galectin-9, which is encoded by LGALS9, was identified as the ligand of Tim-3 and plays a key role in T cell apoptosis. TGFB1 encodes a secreted ligand in the transforming growth factor-beta (TGF-beta) superfamily of proteins. CD86 [20], LGALS9 [21], and TGFB1 [22] play immunosuppressive roles in glioma. Our results showed that C1RL expression exhibited positive relations with CD86, LGALS9, and TGFB1 (Fig. 3.A-L).

**C1RL expression was correlated with reduced tumour purity and increased M2 macrophage infiltration**

The immune response is based on the migration of immune cells. Both tumour purity and the infiltration of 22 types of leukocytes were assessed for each sample in the TCGA datasets. The samples are displayed in order of
their C1RL expression level. Both the immune score and the stromal score exhibited a positive correlation with C1RL expression trends (Fig. 4.A and B, top panels). In addition, tumour purity showed an inverse correlation with C1RL expression trends (Fig. 4.A and B, middle panels). Moreover, C1RL expression was mostly related to the infiltration of M2 macrophages among the 22 types of leukocytes (Fig. 4.A and B, bottom panels).

High expression of C1RL predicted unfavourable survival and therapeutic resistance in glioma

The median expression value of C1RL was used to separate samples into two subgroups. We evaluated the prognostic value of C1RL in the four glioma datasets. The patients with glioma exhibiting higher C1RL expression had significantly shorter survival times than their counterparts in the GSE16011mic, TCGAseq, CGGAmic, and CGGAseq datasets (Fig. 5.A-D). However, the histopathology characteristics in the two subgroups are significantly different (Table S3). The histopathology characteristics may contribute to the survival differences. Next, we compared survival times among GBM patients with different C1RL expression levels in the five datasets. All the survival curves exhibited significant differences (Fig. 5.E-I). Moreover, the effectiveness of well-accepted treatment was evaluated in different C1RL expression groups. The primary GBM patients with lower C1RL expression showed better responses to resection, radiochemotherapy (temozolomide), and standard therapy (Fig. 5.J-L). These results indicate that C1RL may contribute to therapeutic resistance.

Discussion

We analysed the characteristics of C1RL in gliomas from various angles. According to the 2016 WHO classification of glioma, the current diagnosis of glioma is mainly based on the WHO grades and pathology and IDH mutation [23]. Besides, the GBM can be classified into Proneural, Neural, Classical, and Mesenchymal subtypes [24], or primary and secondary GBM [25]. The results showed that the subgroups with worse prognosis always have higher levels of C1RL (Fig. 1). Based on the perspectives that C1RL should have similar biological functions with C1RL-associated genes, we tried to explore the biological function of C1RL with C1RL-associated genes. The GO analyses showed that C1RL-associated genes enriched in immune related biological functions. But we still unsure whether C1RL promotes anti-tumour immune response or suppress it. Given that CD86 [20], LGALS9 [21], and TGFB1 [22] play immunosuppressive roles in glioma, we further investigated the expressing relationship between C1RL and these immunosuppressive genes. Besides, low

Fig. 3 The correlations of C1RL with immunosuppressive genes. The correlations of C1RL with CD86 (a-d), LGALS9 (e-h), and TGFB1 (i-l) in TCGAmic, TCGAseq, CGGAmic, and CGGAseq.
tumour purity [26] and high M2 macrophages infiltration [27] were reported to promote glioma progression. So, we also analysed the relationships between C1RL and glioma purity and leukocyte infiltration.

C1RL is a negative biomarker for glioma prognosis. C1RL had not been mentioned in cancer until a report indicating significant associations of non-Hodgkin lymphoma and diffuse large B-cell lymphoma with the C1RL gene in 2012 [15]. In recent years, C1RL has been reported to be a prognostic biomarker in hepatocellular carcinoma [13] and renal cell cancer [14]. Our results showed evidence that C1RL is highly expressed in glioma samples and predicts a poor prognosis. GBM is WHO grade IV glioma and has the worst prognosis of glioma types, with a median overall survival time of 14.6 months [28]. C1RL expression was always higher in GBM than in LGG (Fig. 1A-H). Unsupervised transcriptomic analysis revealed that of the GBM subtypes, mesenchymal GBM has the worst survival [29]. C1RL expression was higher in mesenchymal GBM than in other GBM subgroups (Fig. 1I-L). Secondary GBM progresses from LGG within 5–10 years of diagnosis and is accompanied by a better prognosis than primary GBM [25]. Secondary GBM exhibited less C1RL expression than primary GBM (Fig. 1M and N). Patients with IDH1-mut glioma have a better outcome than those with IDH1-wt glioma [30]. Relatively low C1RL expression was found in both IDH1-mut LGG and IDH1-mut GBM (Fig. 1O-Q). C1RL not only predicts more advanced malignancy but also worse overall survival in glioma. Due to the distinct outcomes of the glioma subgroups, the differences in C1RL expression in different histopathological subgroups (Fig. 1A-H) may contribute to the observed differences in survival. We further investigated survival differences between the high C1RL expression group and the low C1RL expression group in GBM and even primary GBM. The results confirmed that the expression level of C1RL was a survival indicator in primary GBM (Fig. 5F-I). Resection following chemoradiation is a well-accepted strategy for primary GBM patients. Considering the effects of variant therapies, C1RL may play a role in therapeutic resistance (Fig. 5J-L). Overall, our results indicate that C1RL is a biomarker of poor outcomes in glioma patients.

C1RL probably plays an important role in glioma immunosuppression. The C1RL protein is confirmed to be
an active form of serine hydrolase [17] and cleaves pro-
haptoglobin and pro-C1s into their active forms [18, 19].
On the one hand, due to the suppression of lymphocyte
function by haptoglobin [31], C1RL may modulate immu-
nsuppression in glioma by releasing active haptoglo-
bin. On the other hand, the association of C1s with C1r
and C1q, following ligand recognition, triggers the acti-
vation of the classical complement pathway [32]. C1q
plays a fundamental role in the pathogenesis of glioma
[33]. C1RL may trigger the classical complement path-
way by activating C1s and thus contribute to the patho-
genesis of glioma. In addition, accumulated evidence
shows that C1RL expression is upregulated during in-
flammation [34, 35]. GO analyses of C1RL-associated
genes revealed that they were mainly enriched in the
biological processes of the immune response, inflam-
atory response, IFN-γ mediated signalling pathway, and
innate immune response (Fig. 2.A-D). Furthermore,
C1RL exhibited positive correlations with immunosup-
pressive markers (Fig. 3.A-L).
C1RL expression was correlated with leukocyte infil-
tration, especially M2 macrophage infiltration. Tumour
purity was proposed as an important factor in glioma.
Low purity cases were independently associated with
poor prognosis [26]. Glioma evolution is associated with
immunological changes in the microenvironment [29].
M2 macrophages promotes glioma growth [27]. The
ESTIMATE algorithm is a well-accepted method to pre-
dict the tumour purity in genomic and transcriptomic
studies [36, 37]. Besides, CIBERSORT algorithm, also
known as in silico flow cytometry, was developed to ac-
curately assess the infiltration of many leukocyte subsets
in bulk tumour samples, along with a signature genes file
that enumerates the genes that define the signature ex-
pression profile for each immune cell [38]. The CIBER-
SORT algorithm can be access online (https://cibersort.
stanford.edu/) to characterize cell composition of com-
plex tissues from their gene expression profiles. In this
study, both ESTIMATE algorithm and CIBERSORT al-
gorithm were used to further assess the relationships be-
tween C1RL mRNA expression and 22 different immune
cell populations. Increased amounts of immune cells,
especially M2 macrophages, migrated into glioma tumours
with relatively high C1RL expression (Fig. 4.A and B).
All these results are consistent with the hypothesis that
C1RL plays an immunosuppressive role in glioma.

Conclusions
In conclusion, we analysed the immunological and clini-
copathological characteristics of C1RL in 2120 glioma

![Fig. 5 Survival differences according to C1RL expression in glioma. High C1RL expression predicted shortened overall survival for glioma patients (a) and primary glioma patients (b-d). High C1RL expression predicted shortened overall survival for GBM patients (e) and primary GBM patients (f-i). Primary GBM with high C1RL expression showed increased resistance to radiochemotherapy (j), resection (k), and standard therapy (l).](https://cibersort.stanford.edu/)
patients from five datasets. The results indicate that CIRL is a negative biomarker for the patients with glioma. Furthermore, CIRL probably plays an immunosuppressive role in the pathogenesis of glioma by triggering the activation of haptoglobin and C1s.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12885-020-07436-6.

Additional file 1 Table S1. Clinical information of 2143 patients from the different datasets.

Additional file 2 Table S2. CIRL associated genes.

Additional file 3 Table S3. CIRL and WHO grade and glioma histopathology.

Acknowledgements

Not Applicable.

Authors’ contributions

XY and JW participated the design of this study. JW and LT performed the statistical analysis and drafted the manuscript. GL and HW carried out data acquisition. LZ edited the tables, the figures, and the manuscript. All authors have read and approved the final manuscript.

Funding

This work was supported by grants from the Beijing-Tianjin-Hebei Basic Research Cooperation Project (No. 19JCZDJC64200) and the State Scholarship Fund from China Scholarship Council (No. 201806940031).

Availability of data and materials

The microarray dataset of 539 samples (TCGAmic) and the RNA sequencing dataset of 702 samples (TCGAseq) were downloaded from The Cancer Genome Atlas (TCGA, https://xenabrowser.net). The microarray dataset of 301 samples (CCGAgmic) and the RNA sequencing dataset of 325 samples (CCGAgseq) were downloaded from The Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn/). The microarray dataset of 276 samples (GSE16011mic) was downloaded from Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). The survival analysis of GSE16011mic dataset was conducted in R2 (https://hgserver1.amc.nl/cgi-bin/r2/main.cgi).

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Tianjin Medical University General Hospital.

Consent for publication

Informed consent was obtained from all participants for publication.

Competing interests

The authors declare no competing interests.

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