proceedings

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Proceeding Paper

The Immune Tumor Microenvironment in Gliomas: May CITED2 Play a Role? †

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† Presented at the VII Poster Sunset Session—ESSUAlg 2024, Faro, Portugal, 30 January 2024.

Abstract: Gliomas are the most common brain cancers, resulting from transformed glial cells. CITED2 is a co-transcriptional regulator previously implicated in several types of cancer, affecting both cell-intrinsic processes and the microenvironment. Because in breast cancer it was shown to contribute to the recruitment of macrophages and their polarization to an immunosuppressive phenotype, a potential similar role was explored in gliomas. By analyzing publicly available databases using a set of bioinformatics tools, it was found that CITED2 is overexpressed in higher-grade gliomas and contributes to an adverse prognosis. In addition, CITED2 expression correlates with macrophage infiltration and a M2 phenotype.

Keywords: glioma; immune cells; tumor microenvironment

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Citation: Fernandes, M.T. The Immune Tumor Microenvironment in Gliomas: May CITED2 Play a Role? *Proceedings* **2024**, *99*, 3. https://doi.org/10.3390/proceedings2024099003

Academic Editors: Ana Luísa De Sousa-Coelho, M. Dulce Estêvão, Margarida Espírito-Santo, Luis Braz and Tânia Nascimento

Published: 11 April 2024

1. Introduction

Gliomas are the most common malignant tumors of the central nervous system [1,2]. Of these, 50% are classified as glioblastoma (GBM), a highly aggressive glioma (grade 4) with a poor prognosis [3].

The current standard of care for GBM is resection surgery, followed by radiotherapy and chemotherapy with temozolomide. Lomustine and bevacizumab are generally used when the tumor progresses, but these therapeutic approaches are marked by tumor resistance and do not lead to an increase in overall survival [2]. More recently, immunotherapies have revolutionized the treatment of cancer, but they have been shown to be ineffective in GBM, at least as monotherapy [4]. This appears to be mainly due to the existence of the blood–brain barrier and the characteristic immunosuppressive tumor microenvironment (TME), which is still poorly characterized [5].

Among the cells that make up the TME, GBM-associated macrophages/microglia (GAM) constitute the most abundant cell population [6,7]. In this context, GAMs present a pro-tumor/immunosuppressive M2 phenotype, to the detriment of M1 with pro-inflammatory and anti-tumor activity [8], which contributes to the immunosuppressive microenvironment.

The protein CITED2 (CBP/p300-interacting transactivator with ED-rich tail 2) is a transcriptional modulator with key roles in processes such as self-renewal and cell differentiation [9]. It has also been implicated in the development of diverse types of cancer, with either pro-tumorigenic and anti-tumorigenic roles [9,10]. Nevertheless, a direct role for CITED2 in gliomas, whether cell intrinsic or microenvironmental, has not previously been reported.

Within the scope of TME composition, CITED2 has been shown to induce CCL20 expression and macrophage recruitment to breast cancer tumors, [11] and, in macrophages, it restricts NF-κB activation by negatively interfering with the expression of pro-inflammatory mediators [12,13]. Therefore, CITED2 may be involved in the recruitment and polarization of macrophages, favoring their immunosuppressive effects. Modeling its expression could

be a potential strategy to reprogramming GAMs to a pro-inflammatory phenotype, which has been indicated as a promising antitumor strategy for glioblastoma [14].

2. Materials and Methods

Data from the publicly available databases Genotype–Tissue Expression (GTEx) Data Set and The Cancer Genome Atlas (TCGA), including the lower-grade glioma (LGG) and glioblastoma (GBM) cohorts, were analyzed [15]. RNA sequencing (Illumina HiSeq) and survival data were accessed through the UCSC Xena platform [16] (https://xenabrowser.net/heatmap/, accessed on 5 January 2024). Differential expression analyses between LGG vs. normal tissue and GBM vs. normal tissue were performed using the GEPIA2 Gene Expression Profiling Interactive Analysis platform [17] (http://gepia2.cancer-pku.cn/#degenes, accessed on 5 January 2024). Gene set enrichment (GSEA) analyses were performed using Enrichr [18–20] (https://maayanlab.cloud/Enrichr/, accessed on 5 January 2024). TIMER2.0 web server [21–23] (http://timer.cistrome.org/, accessed on 5 January 2024) was used to analyze tumor-infiltrating immune cells in LGG and GBM.

Statistical analyses were performed using Prism 5 (GraphPad Software 8.4.0, La Jolla, CA, USA). Statistical tests were used as indicated in figure legends, and a $p < 0.05$ was considered statistically significant.

3. Results

3.1. CITED2 Is Significantly More Expressed in GBM Than in LGG

To determine whether CITED2 expression differs between different glioma stages, data from TCGA's lower-grade glioma (grades 2 and 3) and glioblastoma (grade 4) were compared. CITED2 levels were found to be significantly increased in GBM (Figure 1a). Higher CITED2 expression was also associated with an adverse prognosis in glioma (Figure 1b).

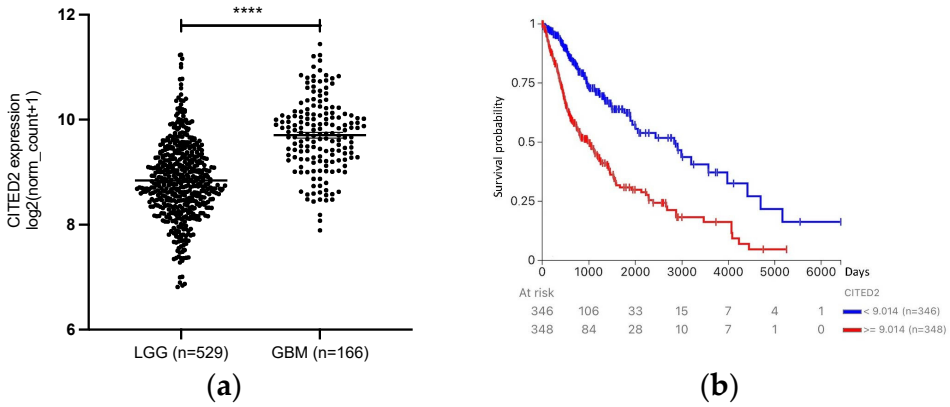

Figure 1. CITED2 expression in lower-grade gliomas (LGG) and glioblastoma (GBM) and the impact on overall survival. (**a**) CITED2 expression by RNA seq (IlluminaHiSeq 2000 RNA sequencing dataset) in LGG and GBM. Results are expressed as the mean ± SEM. ****, $p < 0.0001$, unpaired *t*-test. (**b**) Kaplan–Meier survival curves for patients with CITED2 higher vs. lower expression. ****, $p < 0.0001$, log-rank test. Data derived from the lower-grade glioma and glioblastoma (GBMLGG) cohort of the The Cancer Genome Atlas (TCGA).

CITED2 was previously shown to be expressed in cancer cells but also in tumor-infiltrating cells, like immune cells or endothelial cells [24]. Therefore, to explore the biological processes enriched in LGG and GBM vs. normal tissues, the differential expression was assessed and the top 50 genes with the highest fold-change were analyzed. Interestingly, genes involved in immunological processes were significantly more expressed in GBM than in normal tissues, differing from LGG. In accordance with this result, GBM was previously shown to present higher immune infiltration than LGG [25].

3.2. CITED2 Expression Correlates with Macrophage Infiltration

To evaluate whether CITED2 expression can influence macrophage infiltration, the TIMER2.0 tool was used. As expected, CITED2 expression was correlated with macrophage infiltration, both M1 and M2 (Table 1). In LGG, only M2 macrophage infiltration was correlated with CITED2 expression (Table 2).

Table 1. Correlation between CITED2 expression and macrophage infiltration in GBM (n = 153).

Macrophage Subtype	Rho	p Value
All	0.181	$p < 0.05$ [1]
M1	0.253	$p < 0.05$ [1]
M2	0.266	$p < 0.01$ [2]

[1] TIMER algorithm; [2] CIBERSORT-ABS algorithm.

Table 2. Correlation between CITED2 expression and macrophage infiltration in LGG (n = 516).

Macrophage Subtype	Rho	p Value
All	0.209	$p < 0.05$ [1]
M1	−0.011	ns [2]
M2	0.143	$p < 0.05$ [3]

[1] TIMER algorithm; [2] QUANTISEC algorithm; [3] CIBERSORT-ABS algorithm; ns, non-significant.

Finally, to explore the clinical relevance of macrophage infiltration, a multivariable Cox proportional hazard model was used. Although in GBM, only age and macrophage infiltration were shown to be relevant for survival (age, HR 1.031, $p < 0.001$; macrophage infiltration XCELL, HR 290.680, $p = 0.047$; CITED2 expression, HR 1.082, $p = 0.579$), regarding LGG, the same two variables and CITED2 expression were shown to significantly impact survival (age, HR 1.057, $p < 0.001$; macrophage infiltration TIMER, HR 9.675, $p < 0.001$; CITED2 expression, HR 1.538, $p < 0.001$), leading to an adverse prognosis.

Altogether, these results suggest that CITED2 expression may be relevant in attracting macrophages and polarizing them to an M2 phenotype in an early phase of glioma development.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: These data were derived from the following resources available in the public domain: [UCSC Xena platform (https://xenabrowser.net/heatmap/), GEPIA2 Gene Expression Profiling Interactive Analysis platform (http://gepia2.cancer-pku.cn/#degenes), TIMER2.0 web server (http://timer.cistrome.org/)].

Acknowledgments: The author would like to thank the contributors of UCSC XENA, GEPIA2, Appyters, and TIMER2.0 for the availability of the data and tools.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Rong, L.; Li, N.; Zhang, Z. Emerging therapies for glioblastoma: Current state and future directions. *J. Exp. Clin. Cancer Res.* **2022**, *41*, 142. [CrossRef] [PubMed]
2. Weller, M.; Wick, W.; Aldape, K.; Brada, M.; Berger, M.; Pfister, S.M.; Nishikawa, R.; Rosenthal, M.; Wen, P.Y.; Stupp, R.; et al. Glioma. *Nat. Rev. Dis. Primer* **2015**, *1*, 15017. [CrossRef] [PubMed]
3. Louis, D.N.; Perry, A.; Wesseling, P.; Brat, D.J.; Cree, I.A.; Figarella-Branger, D.; Hawkins, C.; Ng, H.K.; Pfister, S.M.; Reifenberger, G.; et al. The 2021 WHO Classification of Tumors of the Central Nervous System: A summary. *Neuro-Oncology* **2021**, *23*, 1231–1251. [CrossRef] [PubMed]
4. Huang, B.; Li, X.; Li, Y.; Zhang, J.; Zong, Z.; Zhang, H. Current Immunotherapies for Glioblastoma Multiforme. *Front. Immunol.* **2020**, *11*, 603911. [CrossRef] [PubMed]

5. Preddy, I.; Nandoliya, K.; Miska, J.; Ahmed, A.U. Checkpoint: Inspecting the barriers in glioblastoma immunotherapies. *Semin. Cancer Biol.* **2022**, *86 Pt 3*, 473–481. [CrossRef] [PubMed]
6. Quail, D.F.; Joyce, J.A. The Microenvironmental Landscape of Brain Tumors. *Cancer Cell* **2017**, *31*, 326–341. [CrossRef]
7. Chen, Z.; Feng, X.; Herting, C.J.; Garcia, V.A.; Nie, K.; Pong, W.W.; Rasmussen, R.; Dwivedi, B.; Seby, S.; Wolf, S.A.; et al. Cellular and Molecular Identity of Tumor-Associated Macrophages in Glioblastoma. *Cancer Res.* **2017**, *77*, 2266–2278. [CrossRef]
8. Wei, J.; Chen, P.; Gupta, P.; Ott, M.; Zamler, D.; Kassab, C.; Bhat, K.P.; Curran, M.A.; de Groot, J.F.; Heimberger, A.B. Immune biology of glioma-associated macrophages and microglia: Functional and therapeutic implications. *Neuro-Oncology* **2020**, *22*, 180–194. [CrossRef] [PubMed]
9. Fernandes, M.T.; Calado, S.M.; Mendes-Silva, L.; Bragança, J. CITED2 and the modulation of the hypoxic response in cancer. *World J. Clin. Oncol.* **2020**, *11*, 260–274. [CrossRef]
10. An, B.; Ji, X.; Gong, Y. Role of CITED2 in stem cells and cancer. *Oncol. Lett.* **2020**, *20*, 107. [CrossRef]
11. Jayaraman, S.; Doucet, M.; Kominsky, S.L. CITED2 attenuates macrophage recruitment concordant with the downregulation of CCL20 in breast cancer cells. *Oncol. Lett.* **2018**, *15*, 871–878. [CrossRef] [PubMed]
12. Pong Ng, H.; Kim, G.D.; Ricky Chan, E.; Dunwoodie, S.L.; Mahabeleshwar, G.H. CITED2 limits pathogenic inflammatory gene programs in myeloid cells. *FASEB J.* **2020**, *34*, 12100–12113. [CrossRef]
13. Kim, G.-D.; Das, R.; Rao, X.; Zhong, J.; Deiuliis, J.A.; Ramirez-Bergeron, D.L.; Rajagopalan, S.; Mahabeleshwar, G.H. CITED2 Restrains Proinflammatory Macrophage Activation and Response. *Mol. Cell. Biol.* **2018**, *38*, e00452-17. [CrossRef] [PubMed]
14. Xuan, W.; Lesniak, M.S.; James, C.D.; Heimberger, A.B.; Chen, P. Context-Dependent Glioblastoma-Macrophage/Microglia Symbiosis and Associated Mechanisms. *Trends Immunol.* **2021**, *42*, 280–292. [CrossRef] [PubMed]
15. Brennan, C.W.; Verhaak, R.G.W.; McKenna, A.; Campos, B.; Noushmehr, H.; Salama, S.R.; Zheng, S.; Chakravarty, D.; Sanborn, J.Z.; Berman, S.H.; et al. The somatic genomic landscape of glioblastoma. *Cell* **2013**, *155*, 462–477. [CrossRef]
16. Goldman, M.J.; Craft, B.; Hastie, M.; Repečka, K.; McDade, F.; Kamath, A.; Banerjee, A.; Luo, Y.; Rogers, D.; Brooks, A.N.; et al. Visualizing and interpreting cancer genomics data via the Xena platform. *Nat. Biotechnol.* **2020**, *38*, 675–678. [CrossRef] [PubMed]
17. Tang, Z.; Kang, B.; Li, C.; Chen, T.; Zhang, Z. GEPIA2: An enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res.* **2019**, *47*, W556–W560. [CrossRef] [PubMed]
18. Chen, E.Y.; Tan, C.M.; Kou, Y.; Duan, Q.; Wang, Z.; Meirelles, G.V.; Clark, N.R.; Ma'Ayan, A. Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinform.* **2013**, *14*, 128. [CrossRef] [PubMed]
19. Kuleshov, M.V.; Jones, M.R.; Rouillard, A.D.; Fernandez, N.F.; Duan, Q.; Wang, Z.; Koplev, S.; Jenkins, S.L.; Jagodnik, K.M.; Lachmann, A.; et al. Enrichr: A comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res.* **2016**, *44*, W90–W97. [CrossRef]
20. Xie, Z.; Bailey, A.; Kuleshov, M.V.; Clarke, D.J.B.; Evangelista, J.E.; Jenkins, S.L.; Lachmann, A.; Wojciechowicz, M.L.; Kropiwnicki, E.; Jagodnik, K.M.; et al. Gene Set Knowledge Discovery with Enrichr. *Curr. Protoc.* **2021**, *1*, e90. [CrossRef]
21. TIMER2.0 for Analysis of Tumor-Infiltrating Immune Cells—PubMed. Available online: https://pubmed.ncbi.nlm.nih.gov/32442275/ (accessed on 22 December 2023).
22. Li, T.; Fan, J.; Wang, B.; Traugh, N.; Chen, Q.; Liu, J.S.; Li, B.; Liu, X.S. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res.* **2017**, *77*, e108–e110. [CrossRef] [PubMed]
23. Li, B.; Severson, E.; Pignon, J.-C.; Zhao, H.; Li, T.; Novak, J.; Jiang, P.; Shen, H.; Aster, J.C.; Rodig, S.; et al. Comprehensive analyses of tumor immunity: Implications for cancer immunotherapy. *Genome Biol.* **2016**, *17*, 174. [CrossRef] [PubMed]
24. Fox, S.B.; Bragança, J.; Turley, H.; Campo, L.; Han, C.; Gatter, K.C.; Bhattacharya, S.; Harris, A.L. CITED4 inhibits hypoxia-activated transcription in cancer cells, and its cytoplasmic location in breast cancer is associated with elevated expression of tumor cell hypoxia-inducible factor 1alpha. *Cancer Res.* **2004**, *64*, 6075–6081. [CrossRef] [PubMed]
25. Feng, Q.; Li, L.; Li, M.; Wang, X. Immunological classification of gliomas based on immunogenomic profiling. *J. Neuroinflamm.* **2020**, *17*, 360. [CrossRef]