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RAPID COMMUNICATION

Development and validation of an immunogenic cell death-related prognostic model, and landscape of the tumor immune microenvironment in glioblastoma

The important role of immunogenic cell death (ICD) in many tumors is increasingly being discovered. However, its mechanisms and potential as a biomarker and therapeutic target in glioblastoma (GBM) have not been well studied. We obtained GBM samples from the Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases, as well as the immunotherapy cohort from the IMvigor210 study. We used unsupervised clustering to obtain two ICD-related clusters, corresponding to the ICD-low and ICD-high subtypes respectively, and the tumor immune microenvironment and prognosis of the two subtypes were significantly different. Subsequently, based on the differentially expressed genes of the two subtypes, we constructed an ICD-related prognostic risk score model by LASSO-Cox regression analysis and constructed a nomogram chart, which can accurately predict the survival prognosis of GBM patients. Further analysis showed that risk score was an independent prognostic factor for GBM patients, and risk score was also confirmed to be correlated with mutation information, immune cells, immune-related pathways, and immunotherapy response. Our results suggest that ICD plays a role in the formation of GBM's tumor microenvironment, influencing the development of tumors, and highlighting the potential of ICD-related genes as prognostic biomarkers, therapeutic targets, and immune response indicators for GBM (Fig. 1 and Table S1).

GBM is the most common primary craniocerebral malignant tumor, which is classified as grade IV by the World Health Organization (WHO), with poor survival.[1] Recently, immunotherapy has become the focus of the current treatment of tumors.[2] It is well known that most tumors form a special immunosuppressive tumor microenvironment (TME), especially GBM, to evade the surveillance of the immune system, greatly reducing the anti-tumor effect of drugs.[3] Besides, GBM has extensive genetic heterogeneity, and there is a large difference in patient's responses to the same treatment. In order to make progress in the treatment of GBM, it is necessary to further explore new therapeutic targets. Currently, immunogenic cell death (ICD) has been widely concerned as a special regulatory death form related to immunity, and then ICD-related treatment began to be popularized.[4] ICDs are induced by stress, including viral infections, chemotherapy drugs, certain forms of radiotherapy, and photodynamic therapy. ICD usually mediates the anti-tumor immune response by transforming from non-immunogenic to immunogenic when tumor cells die from external stimuli. In the process of ICD, dead cells produce new epitopes and release damage-associated molecular patterns (DAMPs), recruit antigen-presenting cells, recognize and phagocytose antigens from dead cells, and present them to T cells. Activation of the adaptive immune response, recognition, and clearance of tumor antigens, resulting in a long-lasting anti-tumor immune effect. Therefore, the reasonable and safe application of ICD inducers in tumor therapy is of great significance for activating anti-tumor immunity and producing long-term anti-tumor effects. Furthermore, immune checkpoint inhibitors have been shown to be effective only in subgroups of patients identified by specific biomarkers.[5] This suggests that exploring ICD-related heterogeneity and the tumor microenvironment in GBM may help us to obtain GBM-related biomarkers and new targets for GBM therapy.

In this study, we obtain TCGA-GBM cohort from TCGA and cerebral cortex samples from Genotype-Tissue Expression (GTEx), GSE7696 datasets from GEO, and an IMvigor210 cohort. We extracted a matrix of 34 ICD-related genes in the training set (Table S2). We found that most

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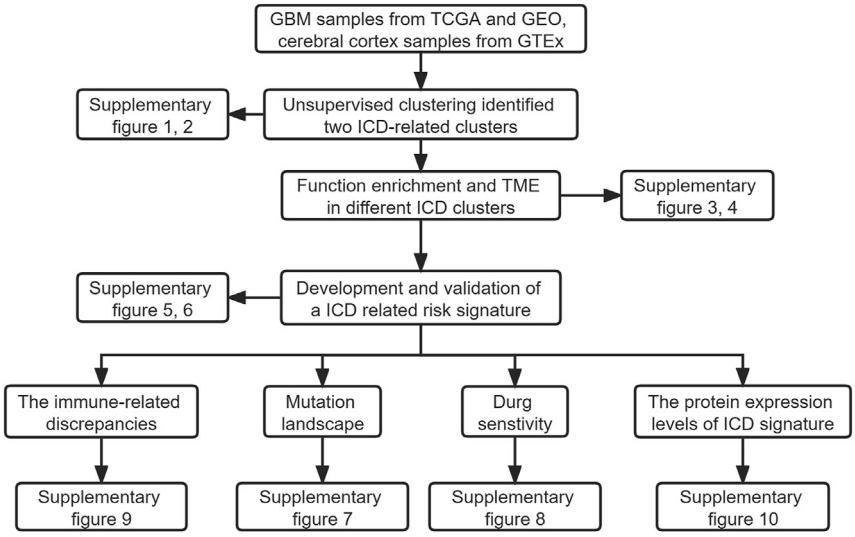

Figure 1 The flow chart shows the main analysis process of our study. First, we obtained glioblastoma (GBM) samples from the Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases, and cerebral cortex samples from GTEx databases. Then, we constructed an immunogenic cell death (ICD)-related GBM stratification model based on ICD-related genes and evaluated the tumor microenvironment and physiological and pathological function of patients with different stratification. Subsequently, we identified ICD-related signatures for the construction of a prognostic scoring model and then evaluated the accuracy and application value of the risk model. Patients were divided into high-risk or low-risk groups based on the median risk score. The somatic mutation, immune landscape, and response to immunotherapy between the two risk groups were assessed. Finally, Human Protein Atlas database was used to obtain the protein expression IHC images of ICD-related genes in our model of GBM patients. We identified 34 ICD-related genes from the previous literature, titled *Immunological metagene signatures derived from immunogenic cancer cell death associate with improved survival of patients with lung, breast or ovarian malignancies: A large-scale meta-analysis*.

ICD-related genes showed higher expression levels in C2 clusters, suggesting the presence of an ICD-high cluster, which had worse survival outcomes (Fig. S1, S2). Then, we obtained 65 DEGs (6 down-regulated and 59 up-regulated genes) between the two clusters. In the GO analysis, 65 DEGs are mainly enriched in the biological processes associated with immunity. In KEGG pathway enrichment analysis, DEGs are mainly involved in the T cell receptor signaling pathway and cytokine receptor (Table S3). In addition, GSEA was performed to identify the difference in signaling pathways activated by the two clusters. We also found that the ICD-high cluster had higher ImmuneScore and less TumorPurity (Fig. S3 and Table S4). The proportion of tumor-infiltrating immune cells in different clusters was varied. Besides, almost all the HLA genes and immune checkpoints had a higher expression in the ICD-high cluster (Fig. S4).

Subsequently, six ICD-related DEGs were considered to be prognostic-related genes. Finally, we constructed the prediction mode, named risk score, with these four DEGs recognized in the LASSO regression analysis. Patients were divided into high- and low-risk groups based on the median patient score in the training set. The survival curve showed the low-risk group had a longer overall survival and progression-free survival (Fig. S5). In addition, a nomogram was constructed to predict the 0.5-, 1-, and 2-OS of GBM patients. The calibration curve and ROC curves confirmed the predictive ability of risk score (Fig. S6).

Mutation landscape low-risk and high-risk subgroups were different. However, there was no significant difference in TMB between the two risk groups, and the TMB showed no significant relationship with the risk score. The survival analysis showed that patients in the high-TMB group had significantly longer survival. Subsequently, we further put risk score into investigation and found that risk score was an independent prognostic factor for GBM patients other than TMB (Fig. S7). Moreover, we evaluated the IC_{50} values for multiple drugs and found significant differences between drugs that were sensitive to low- and high-risk groups (Fig. S8).

We further analyzed the correlation between risk score and tumor immune microenvironment. Besides, the tumor cell stemness score showed a significant negative relationship with the risk score. All immune-related functions and immune cell abundance were significantly different between the high- and low-risk groups. In addition, the TIDE scores were dramatically higher in the high-risk group compared with the low-risk group, indicating that the low-risk group is more likely to benefit from immunotherapy. Interestingly, we found that patients who responded well to immunotherapy were in the low-risk group, consistent with our previous prediction that the low-risk group was more likely to benefit from immunotherapy (Fig. S9). Moreover, HPA database immunohistochemistry results showed that compared with normal cerebral cortex tissues, the expression of IFNA1 and MYD88 in GBM tumor tissues was significantly higher than those in normal tissues (Fig. S10).

ICD is an important part of anti-tumor immunotherapy and its impact on the survival of tumor patients. It is

particularly important to further study and clarify the mechanism of ICD occurrence, explore the impact of ICD on tumor occurrence and progression, and identify ICD-related characteristics for tumor therapy and even the development of tumor vaccines. Based on the 34 ICD-related genes, we successfully distinguished GBM samples into two patterns of ICD expression patterns and found significant differences in the immune microenvironment between these two patterns. Subsequently, based on the DEGs between the two ICD patterns, we finally identified 4 ICD-related signatures and constructed an ICD-related risk score model with good diagnostic ability. Furthermore, the risk score was strongly correlated with the immune microenvironment, drug sensitivity, and tumor stemness scores. This study further clarified the possible mechanism of ICD in GBM and demonstrated the relationship between ICD and GBM prognosis and immunotherapy response. This study provides a new idea for the further development of ICD inducers and promotes the development of precise individualized immunotherapy for GBM.

Although our study has some important findings, we must acknowledge that it has certain limitations. First, our training and validation cohorts were created from a small sample of GBM patients in TCGA and GEO databases. Further large-scale prospective clinical studies are warranted to confirm the predictive significance of the prognostic features of this study. In addition, our model building and correlation analysis are based on bioinformatics studies, and although we found some experimental evidence in HPA databases and published studies, further *in vitro* and *in vivo* studies and mechanistic studies are still needed in the future, which is our future research plan.

Conflict of interests

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.04.032.

References

1. Chen J, Han P, Dahiya S. Glioblastoma: changing concepts in the WHO CNS_5 classification. *Indian J Pathol Microbiol*. 2022; 65(Supplement):S24–S32.
2. Lim M, Xia Y, Bettegowda C, et al. Current state of immunotherapy for glioblastoma. *Nat Rev Clin Oncol*. 2018;15(7): 422–442.
3. Quail DF, Joyce JA. The microenvironmental landscape of brain tumors. *Cancer Cell*. 2017;31(3):326–341.
4. Ahmed A, Tait SWG. Targeting immunogenic cell death in cancer. *Mol Oncol*. 2020;14(12):2994–3006.
5. Zhou J, Wang G, Chen Y, et al. Immunogenic cell death in cancer therapy: present and emerging inducers. *J Cell Mol Med*. 2019; 23(8):4854–4865.

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