Programmed death ligand 1 in glioblastoma

Combination immunotherapy holds promise for improving survival in responsive glioblastoma (GBM) patients. Programmed death-ligand 1 (PD-L1) expression in immune microenvironment (IME) is the most important predictive biomarker for immunotherapy. Due to the heterogeneous distribution of PD-L1, post-operative histopathology fails to accurately capture its expression in residual tumors, making intra-operative diagnosis crucial for GBM treatment strategies. However, the current methods for evaluating the expression of PD-L1 are still time-consuming.

Objective: To overcome the PD-L1 heterogeneity and enable rapid, accurate, and label-free imaging of PD-L1 expression level in GBM IME at the tissue level.

Methods: We proposed a novel intra-operative diagnostic method, Machine Learning Cascade (MLC)-based Raman histopathology, which uses a coordinate localization system (CLS), hierarchical clustering analysis (HCA), support vector machine (SVM), and similarity analysis (SA). This method enables visualization of PD-L1 expression in glioma cells, CD8+ T cells, macrophages, and normal cells in addition to the tumor/normal boundary. The study quantified PD-L1 expression levels using the tumor proportion, combined positive, and cellular composition scores (TPS, CPS, and CCS, respectively) based on Raman data. Furthermore, the association between Raman spectral features and biomolecules was examined biochemically.

Results: The entire process from signal collection to visualization could be completed within 30 minutes. In an orthotopic glioma mouse model, the MLC-based Raman histopathology demonstrated a high average accuracy (0.990) for identifying different cells and exhibited strong concordance with multiplex immunofluorescence (84.31%) and traditional pathologists' scoring ($R2 \geq 0.9$). Moreover, the peak intensities at 837 and 874 cm-1 showed a positive linear correlation with PD-L1 expression level.

Conclusions: This study introduced a new and extendable diagnostic method to achieve rapid and accurate visualization of PD-L1 expression in GBM IMB at the tissular level, leading to great potential in GBM intraoperative diagnosis for guiding surgery and post-operative immunotherapy [1)].

Programmed death-ligand 1 (PD-L1) ensures that tumor cells escape T-cell-mediated tumor immune surveillance. However, gliomas are characteristic of the low immune response and high-resistance therapy, it is necessary to understand molecular regulatory mechanisms in glioblastoma, especially the limited regulation of PD-L1 expression. Herein, Long et al. show that low expression of AP-2α is correlated with high expression of PD-L1 in high-grade glioma tissues. AP-2α binds directly to the promoter of the CD274 gene, not only inhibits the transcriptional activity of PD-L1 but enhances endocytosis and degradation of PD-L1 proteins. Overexpression of AP-2α in gliomas enhances CD8+ T cell-mediated proliferation, effector cytokine secretion, and cytotoxicity in vitro. Tfap2a could increase the cytotoxic effect of Cd8+ T cells in CT26, B16F10, and GL261 tumor-immune models, improve anti-tumor immunity, and promote the efficacy of anti-PD-1 therapy. Finally, the EZH2/H3K27Me3/DNMT1 complex mediates the methylation modification of AP-2α gene and maintains low expression of AP-2α in gliomas. 5-Aza-dC (Decitabine) treatment combines with anti-PD-1 immunotherapy to efficiently suppress the progression of GL261 gliomas. Overall, these data support a mechanism of epigenetic modification of AP-2α that contributes to tumor immune evasion, and reactivation of AP-2α synergizes with anti-PD-1 antibodies to increase antitumor efficacy, which may be a broadly applicable strategy in solid tumors [2)]

Reports of programmed death ligand 1 (PD-L1) expression in glioblastoma are highly variable (ranging from 6% to 88%) and its role as a prognostic marker has yielded conflicting results.

Data points to a putative role for PD-L1 expression in glioblastoma biology, which correlates to poor patient overall survival, as well as with a general systemic inflammatory status and immunosuppression [3].

A 5% PD-L1 expression cut-off identified a subset of glioblastoma that is associated with a worse clinical outcome. This association remained significant within the newly defined IDH wildtype classification. These findings could have implications for patient stratification in future clinical trials of PD-1/PD-L1 blockade [4].

For patients receiving Dendritic cell vaccine adjuvant therapy, better outcomes are predicted in patients with younger age, with TILs or PBMCs with lower PD-1+/CD8+ ratio, with gross tumor resection, and receiving CCRT [5].

In a retrospective cohort of 115 consecutive patients with Glioblastoma, PD-L1 expression was determined using immunohistochemistry (IHC). Membranous and fibrillary PD-L1 staining of any intensity in > 5% neoplastic cells and tumour infiltrating immune cells (TIIs) was considered positive staining. In addition, isocitrate dehydrogenase 1 (IDH-1) (R132H) expression and cluster of differentiation 3 (CD3)-positive T-cell infiltration were investigated using IHC. O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation assay and fluorescence in situ hybridization (FISH) for the assessment of 1p/19q deletion were performed. Expression of PD-L1 in tumour cells and TIIs was found in 37 (32.2%) and 6 (5.2%) patients, respectively. Kaplan-Meier analysis indicated that PD-L1 expression in tumour cells was significantly associated with poor overall survival (OS) ($P = 0.017$), though multivariate Cox analysis did not confirm this association (hazard ratio 1.204; $P = 0.615$). PD-L1 expression in TIIs did not correlate with the patient prognosis ($P = 0.545$). In addition, MGMT methylation and IDH-1 (R132H) expression were associated with a better prognosis ($P < 0.001$ and $P = 0.024$, respectively). The expression of PD-L1 was associated with CD3-positive T-cell infiltration ($P < 0.001$), and IDH-1 wild type status ($P = 0.008$). A deeper insight into PD-L1 expression could help to ensure the success of future immunotherapy in Glioblastoma. Our study suggested that PD-L1 target therapy might be beneficial for PD-L1-expressing Glioblastoma patients with a poor prognosis [6].

Immunotherapies for glioblastoma multiforme including PD-1/PD-L1 inhibition are currently tested in ongoing clinical trials. The purpose of a study was to investigate the molecular background of PD-L1 expression in glioblastoma multiforme and to find associated pathway activation and genetic alterations. Heiland et al., show that PD-L1 is up-regulated in IDH1/2 wildtype glioblastoma multiforme compared to lower-grade gliomas. In addition, a strong association of PD-L1 with the mesenchymal expression subgroup was observed. Consistent with that, NF1 mutation and corresponding activation of the MAPK pathway was strongly connected to PD-L1 expression. The findings may explain different

response to PD-L1 inhibition of patients in ongoing trials and may help to select patients that may profit of immunotherapy in the future [7].

1)
Zhou QQ, Guo J, Wang Z, Li J, Chen M, Xu Q, Zhu L, Xu Q, Wang Q, Pan H, Pan J, Zhu Y, Song M, Liu X, Wang J, Zhang Z, Zhang L, Wang Y, Cai H, Chen X, Lu G. Rapid visualization of PD-L1 expression level in glioblastoma immune microenvironment via machine learning cascade-based Raman histopathology. J Adv Res. 2023 Dec 8:S2090-1232(23)00377-6. doi: 10.1016/j.jare.2023.12.002. Epub ahead of print. PMID: 38072311.
2)
Long S, Huang G, Ouyang M, Xiao K, Zhou H, Hou A, Li Z, Zhong Z, Zhong D, Wang Q, Xiang S, Ding X. Epigenetically modified AP-2α by DNA methyltransferase facilitates glioma immune evasion by upregulating PD-L1 expression. Cell Death Dis. 2023 Jun 17;14(6):365. doi: 10.1038/s41419-023-05878-x. PMID: 37330579.
3)
Noronha C, Ribeiro AS, Taipa R, Leitão D, Schmitt F, Reis J, Faria C, Paredes J. PD-L1 tumor expression is associated with poor prognosis and systemic immunosuppression in glioblastoma. J Neurooncol. 2022 Jan 23. doi: 10.1007/s11060-021-03907-3. Epub ahead of print. PMID: 35066764.
4)
Pratt D, Dominah G, Lobel G, Obungu A, Lynes J, Sanchez V, Adamstein N, Wang X, Edwards NA, Wu T, Maric D, Giles AJ, Gilbert MR, Quezado M, Nduom EK. Programmed Death Ligand 1 Is a Negative Prognostic Marker in Recurrent Isocitrate Dehydrogenase-Wildtype Glioblastoma. Neurosurgery. 2018 Jul 12. doi: 10.1093/neuros/nyy268. [Epub ahead of print] PubMed PMID: 30011045.
5)
Jan CI, Tsai WC, Harn HJ, Shyu WC, Liu MC, Lu HM, Chiu SC, Cho DY. Predictors of Response to Autologous Dendritic Cell Therapy in Glioblastoma Multiforme. Front Immunol. 2018 May 29;9:727. doi: 10.3389/fimmu.2018.00727. eCollection 2018. PubMed PMID: 29910795; PubMed Central PMCID: PMC5992384.
6)
Lee KS, Lee K, Yun S, Moon S, Park Y, Han JH, Kim CY, Lee HS, Choe G. Prognostic relevance of programmed cell death ligand 1 expression in glioblastoma. J Neurooncol. 2018 Feb;136(3):453-461. doi: 10.1007/s11060-017-2675-6. Epub 2017 Nov 16. PubMed PMID: 29147863.
7)
Heiland DH, Haaker G, Delev D, Mercas B, Masalha W, Heynckes S, Gäbelein A, Pfeifer D, Carro MS, Weyerbrock A, Prinz M, Schnell O. Comprehensive analysis of PD-L1 expression in glioblastoma multiforme. Oncotarget. 2017 Feb 2. doi: 10.18632/oncotarget.15031. [Epub ahead of print] PubMed PMID: 28178682.