

Additional file 4: Supplementary Materials and Methods

Article Title:

Enhanced B7-H4 expression in gliomas with low PD-L1 expression identifies super-cold tumors

Authors:

Di Chen¹, Gaopeng Li², Chunxia Ji³, Qiqi Lu³, Ying Qi¹, Chao Tang^{1,3}, Ji Xiong⁴, Jian Hu⁵, Fatma Betul Aksoy Yasar⁵, Yan Zhang², Dave S.B. Hoon⁶, Yu Yao¹, Liangfu Zhou^{1,3}

Affiliations:

- 1 Department of Neurosurgery, Huashan hospital, Fudan University, Shanghai, China
- 2 Shenzhen Key Laboratory of Marine Bioresources and Ecology, Brain Disease and Big Data Research Institute, College of Life Sciences & and Oceanography, Shenzhen University, Shenzhen, Guangdong, China
- 3 Neurosurgical Immunology Laboratory, Neurosurgical Institute of Fudan University, Shanghai, China
- 4 Department of Pathology, Huashan hospital, Fudan University, Shanghai, China
- 5 Department of Cancer Biology, University of Texas MD Anderson Cancer Center, Houston, TX, USA
- 6 Department of Translational Molecular Medicine, John Wayne Cancer Institute, Providence Health Systems, Santa Monica, CA, USA

Supplementary Materials and Methods

H-score analysis for PD-L1 and B7-H4

H-scores were calculated as reported in PD-L1 previously^{1 2}. Briefly, the positive proportion of staining were multiplied by a grading value corresponding to the maximum intensity score to give a H-score ranging from 0 to 300. An H-score threshold of 5 (≥ 5 versus < 5) was determined as reported in previous studies^{3 4} to differentiate from positive and negative staining.

Development and evaluation of B7-H4-overexpressing GL261 cells

The full-length sequence of B7-H4 was cloned into a lentiviral expression vector tagged with GFP (pHBLV-EF1-MCS-P2A-ZsGreen-P2A-Puro). This vector was transfected into 293T cells to package lentivirus. GL261 cells were grown to 70% confluence in 24-well plates and incubated with the lentivirus supernatant for 4h. The overexpression of B7-H4 was confirmed by flow cytometry as follows: the GL261 cells were incubated on ice for 30 minutes with anti-B7-H4 antibody (Alexa Fluor 647-B7-H4, Recombinant Monoclonal Rabbit IgG Clone # 2319B, R&D Systems) and washed twice with FACS buffer and assessed by FACS. The cells showing double-positive with GFP and B7-H4 were determined as hB7-H4/GL261 cells. The GL261 cells transfected with an empty vector (Control/GL261) were designed for negative control.

Correlation analysis between B7-H4 and clinical benefits responses from immunotherapy

We downloaded and analyzed clinical and mRNA data publicly available from melanoma (n=49, GSE91061)⁵ and non-small cell lung cancer (n=21, GSE136961)⁶ who were treated with anti-PD-1 antibodies. The patients were dichotomized as responder and non-responder based on RECIST v1.1. The read counts data of B7-H4 were log₂ normalized and compared between responder and non-responder with t-test.

References

1. Cheng H, Borczuk A, Janakiram M, et al. Wide Expression and Significance of Alternative Immune Checkpoint Molecules, B7 χ and HHLA2, in PD-L1-Negative Human Lung Cancers. *Clinical cancer research* 2018;24(8):1954-64. doi: 10.1158/1078-0432.ccr-17-2924
2. Cheng H, Janakiram M, Borczuk A, et al. HHLA2, a New Immune Checkpoint Member of the B7 Family, Is Widely Expressed in Human Lung Cancer and Associated with EGFR Mutational Status. *Clinical cancer research* 2017;23(3):825-32. doi: 10.1158/1078-0432.ccr-15-3071
3. Schalper KA, Carvajal-Hausdorf D, McLaughlin J, et al. Differential Expression and Significance of PD-L1, IDO-1, and B7-H4 in Human Lung Cancer. *Clinical cancer research* 2017;23(2):370-78. doi: 10.1158/1078-0432.ccr-16-0150
4. Rosenbaum MW, Gigliotti BJ, Pai SI, et al. PD-L1 and IDO1 Are Expressed in Poorly Differentiated Thyroid Carcinoma. *Endocrine pathology* 2018;29(1):59-67. doi: 10.1007/s12022-018-9514-y
5. Riaz N, Havel JJ, Makarov V, et al. Tumor and Microenvironment Evolution during Immunotherapy with Nivolumab. *Cell* 2017;171(4):934-49.e16. doi: 10.1016/j.cell.2017.09.028
6. Hwang S, Kwon AY, Jeong JY, et al. Immune gene signatures for predicting durable clinical benefit of anti-PD-1 immunotherapy in patients with non-small cell lung cancer. *Scientific reports* 2020;10(1):643. doi: 10.1038/s41598-019-57218-9