Additional file 2: Supplementary Tables

Article Title:

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

Authors:

Di Chen[1], Gaopeng Li[2], Chunxia Ji[3], Qiqi Lu[3], Ying Qi[1], Chao Tang[1, 3], Ji Xiong[4], Jian Hu[5], Fatma Betul Aksoy Yasar[5], Yan Zhang[2], Dave S.B. Hoon[6], Yu Yao[1], 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

Table S1. Patient Characteristics in IHC cohort (n=505)

Characteristics	No. of Patients	%
Gender		
Male	295	58.4%
Female	208	41.2%
NA	2	0.4%
Age (Years)		
Median	47	
Range	5-79	
Radiotherapy		
Yes	355	70.3%
No	46	9.1%
NA	104	20.6%
Chemotherapy		
Yes	422	83.6%
No	70	13.9%
NA	13	2.5%
Tumor Location		
Frontal	207	41.0%
Non-frontal	192	38.0%
NA	106	21.0%
EOR		
CR	365	72.3%
ICR	78	15.4%
Biospy	6	1.2%
NA	56	11.1%
Histology		
Oligodendroglioma	86	17.0%
Astrocytoma	185	36.6%
Glioblastoma	234	46.4%
WHO Grade		
WHO II	145	28.7%
WHO III	126	25.0%
WHO IV	234	46.3%
IDH status		
Mutation	155	30.7%
Wild-type	114	22.6%
NA	236	46.7%
TERT status		
Mutation	111	22.0%
Wild-type	158	31.3%
NA	236	46.7%
Chr1p/19q status		
Codeletion	65	12.9%

Non-codeletion	172	34.1%
NA	268	53.0%

Abbreviation: EOR, extent of resection; CR, complete resection; ICR, incomplete resection; NA is data not available.

Table S2. Resource table of antibodies

Antibody	**Species reactivity**	**Source**	**Identifier**	**References (PMID)**
Rabbit monoclonal Anti-B7-H4	Human	Abcam	Cat#ab209242	31223291, 29436630
Rabbit monoclonal Anti-PD-L1	Human	Abcam	Cat#ab205921	30687086, 30808674
Rabbit polyclonal Anti-CD3	Human, Mouse, Rat	Abcam	Cat#ab5690	30467920, 24903021
Rabbit monoclonal Anti-CD163	Human, Mouse, Rat	Abcam	Cat#ab182422	30041684, 30926388

Table S3. Interpretation of *kappa* values for the inter-observer agreement

***kappa* values**	**Levels of agreement**
≤0.2	Poor
0.21 to 0.40	Fair
0.41 to 0.60	Moderate
0.61 to 0.80	Strong
>0.80	Almost perfect

Table S4. *Kappa* values and Spearman's testing for inter-observer agreement (two observers)

Parameters	***Kappa*/ Spearman**	**Agreement**
PD-L1 (High expression and Low expression)	0.694 (*Kappa*)	Strong
B7-H4 (High expression and Low expression)	0.717 (*Kappa*)	Strong
CD3 (1, 2, 3, 4)	0.687 (*Kappa*)	Strong
CD163 (continuous numerical value)	0.956 (Spearman)	/

Table S5. PD-L1/B7-H4 classifier genes extracted from TCGA dataset

Symbol	Class	Description
ADPRH	PD-L1	ADP-ribosylarginine hydrolase
ALOX5	PD-L1	arachidonate 5-lipoxygenase
APOBEC3G	PD-L1	apolipoprotein B mRNA editing enzyme catalytic subunit 3G
ARHGAP25	PD-L1	Rho GTPase activating protein 25
B3GNT5	PD-L1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5
CASP1	PD-L1	caspase 1
CASP8	PD-L1	caspase 8
CAST	PD-L1	calpastatin
CCR5	PD-L1	C-C motif chemokine receptor 5 (gene/pseudogene)
CD274	PD-L1	CD274 molecule
CTBS	PD-L1	chitobiase
CTF1	PD-L1	cardiotrophin 1
CTSZ	PD-L1	cathepsin Z
DCTD	PD-L1	dCMP deaminase
ELK3	PD-L1	ELK3, ETS transcription factor
ERMAP	PD-L1	erythroblast membrane associated protein (Scianna blood group)
ESYT1	PD-L1	extended synaptotagmin 1
GALM	PD-L1	galactose mutarotase
GBP5	PD-L1	guanylate binding protein 5
GCLM	PD-L1	glutamate-cysteine ligase modifier subunit
GNG12	PD-L1	G protein subunit gamma 12
GSAP	PD-L1	gamma-secretase activating protein
HIVEP3	PD-L1	human immunodeficiency virus type I enhancer binding protein 3
IFIH1	PD-L1	interferon induced with helicase C domain 1
IL13RA1	PD-L1	interleukin 13 receptor subunit alpha 1
IQGAP1	PD-L1	IQ motif containing GTPase activating protein 1
LAP3	PD-L1	leucine aminopeptidase 3
LYN	PD-L1	LYN proto-oncogene, Src family tyrosine kinase
MR1	PD-L1	major histocompatibility complex, class I-related
MYOF	PD-L1	myoferlin
NMI	PD-L1	N-myc and STAT interactor
OSBPL3	PD-L1	oxysterol binding protein like 3
P3H2	PD-L1	prolyl 3-hydroxylase 2
PARP14	PD-L1	poly(ADP-ribose) polymerase family member 14
PARP9	PD-L1	poly(ADP-ribose) polymerase family member 9
PCED1B	PD-L1	PC-esterase domain containing 1B
PDCD1LG2	PD-L1	programmed cell death 1 ligand 2
PLEK	PD-L1	pleckstrin
PLS3	PD-L1	plastin 3
PPCS	PD-L1	phosphopantothenoylcysteine synthetase
PPM1M	PD-L1	protein phosphatase, Mg2+/Mn2+ dependent 1M
PTGS1	PD-L1	prostaglandin-endoperoxide synthase 1

PTPRC	PD-L1	protein tyrosine phosphatase, receptor type C
RAB27A	PD-L1	RAB27A, member RAS oncogene family
RASSF5	PD-L1	Ras association domain family member 5
RBM47	PD-L1	RNA binding motif protein 47
RILPL2	PD-L1	Rab interacting lysosomal protein like 2
RNF135	PD-L1	ring finger protein 135
SAMD9L	PD-L1	sterile alpha motif domain containing 9 like
SLC2A10	PD-L1	solute carrier family 2 member 10
SP100	PD-L1	SP100 nuclear antigen
SP140L	PD-L1	SP140 nuclear body protein like
SPATS2L	PD-L1	spermatogenesis associated serine rich 2 like
SWAP70	PD-L1	switching B cell complex subunit SWAP70
TBC1D1	PD-L1	TBC1 domain family member 1
TLR3	PD-L1	toll like receptor 3
TMCO4	PD-L1	transmembrane and coiled-coil domains 4
TMEM109	PD-L1	transmembrane protein 109
TMEM154	PD-L1	transmembrane protein 154
TRIM38	PD-L1	tripartite motif containing 38
UNC93B1	PD-L1	unc-93 homolog B1, TLR signaling regulator
WWTR1	PD-L1	WW domain containing transcription regulator 1
ALCAM	B7-H4	activated leukocyte cell adhesion molecule
AMOTL2	B7-H4	angiomotin like 2
ATF7IP	B7-H4	activating transcription factor 7 interacting protein
C16orf87	B7-H4	chromosome 16 open reading frame 87
CCNJ	B7-H4	cyclin J
CHD7	B7-H4	chromodomain helicase DNA binding protein 7
MARCH8	B7-H4	membrane associated ring-CH-type finger 8
CTTNBP2	B7-H4	cortactin binding protein 2
EFCAB5	B7-H4	EF-hand calcium binding domain 5
EPB41	B7-H4	erythrocyte membrane protein band 4.1
EPHB1	B7-H4	EPH receptor B1
FERMT1	B7-H4	fermitin family member 1
GLCCI1	B7-H4	glucocorticoid induced 1
KLRC3	B7-H4	killer cell lectin like receptor C3
KLRC4-KLRK1	B7-H4	KLRC4-KLRK1 readthrough
MACO1	B7-H4	macoilin 1
MAML2	B7-H4	mastermind like transcriptional coactivator 2
MEX3A	B7-H4	mex-3 RNA binding family member A
MEX3B	B7-H4	mex-3 RNA binding family member B
MKRN3	B7-H4	makorin ring finger protein 3
MYCL	B7-H4	MYCL proto-oncogene, bHLH transcription factor
NKAIN1	B7-H4	sodium/potassium transporting ATPase interacting 1
OLIG2	B7-H4	oligodendrocyte transcription factor 2
OPHN1	B7-H4	oligophrenin 1

P2RX7	B7-H4	purinergic receptor P2X 7
PCDH15	B7-H4	protocadherin related 15
RBPJ	B7-H4	recombination signal binding protein for immunoglobulin kappa J region
SERINC5	B7-H4	serine incorporator 5
SLCO5A1	B7-H4	solute carrier organic anion transporter family member 5A1
SOX4	B7-H4	SRY-box 4
SOX6	B7-H4	SRY-box 6
TMCC1	B7-H4	transmembrane and coiled-coil domain family 1
USP49	B7-H4	ubiquitin specific peptidase 49
VTCN1	B7-H4	V-set domain containing T cell activation inhibitor 1
ZNF74	B7-H4	zinc finger protein 74

Table S6. Association between PD-L1, B7-H4 and clinicopathologic variables in IHC cohort (n=505)

Characteristics	**PD-L1 low**	**PD-L1 high**	**P value**	**B7-H4 low**	**B7-H4 high**	**P value**
Gender						
Male	229	66	0.493	228	67	**0.042**
Female	156	52		176	32	
NA						
Age (Years)						
＜40	124	27	0.053	118	32	0.422
≥40	261	91		285	66	
NA						
Tumor Location						
Frontal	168	39	**0.028**	179	28	0.644
Non-frontal	138	54		169	23	
NA						
EOR						
CR	277	88	0.490	300	65	0.061
ICR	64	14		56	22	
Biospy	5	1		6	0	
NA						
Histology						
Oligodendroglioma	75	11	**0.006**	77	9	**＜0.0001**
Astrocytoma	146	39		128	57	
Glioblastoma	166	68		201	33	
WHO Grade						
WHO II	127	18	**0.001**	124	21	**＜0.0001**
WHO III	94	32		81	45	
WHO IV	166	68		201	33	
IDH status						
Mutation	131	24	**0.003**	106	49	0.883
Wild-type	79	35		77	37	
NA						
TERT status						
Mutation	88	23	0.687	92	19	**＜0.0001**
Wild-type	122	36		90	68	
NA						
Chr1p/19q status						
Codeletion	56	9	0.111	55	10	**0.0007**
Non-codeletion	132	40		106	66	
NA						

Abbreviation: EOR, extent of resection; CR, complete resection; ICR, incomplete resection; NA is data not available.

Table S7. Association between PD-L1/B7-H4 subgroups and clinicopathologic variables in IHC cohort (n=505)

Characteristics	**High-PD-L1**	**High-B7-H4**	**Double-Low**	**Double-High**	**P value**
Gender					
Male	57	58	171	9	0.189
Female	49	29	127	3	
NA					
Age (Years)					
＜40	24	30	94	3	0.251
≥40	82	57	204	9	
NA					
Tumor Location					
Frontal	36	25	143	3	0.164
Non-frontal	51	20	118	3	
NA					
EOR					
CR	81	58	219	7	0.140
ICR	10	18	46	4	
Biospy	1	0	5	0	
NA					
Histology					
Oligodendroglioma	10	8	67	1	**＜0.0001**
Astrocytoma	32	50	96	7	
Glioblastoma	64	29	137	4	
WHO Grade					
WHO II	17	20	107	1	**＜0.0001**
WHO III	25	38	56	7	
WHO IV	64	29	137	4	
IDH status					
Mutation	17	42	89	7	**0.003**
Wild-type	31	33	46	4	
NA					
TERT status					
Mutation	20	16	72	3	**＜0.0001**
Wild-type	28	60	62	8	
NA					
Chr1p/19q status					
Codeletion	8	9	47	1	**0.001**
Non-codeletion	30	56	76	10	
NA					

Abbreviation: EOR, extent of resection; CR, complete resection; ICR, incomplete resection; NA is data not available.

Table S8. Top 50 GO enrichment terms of PD-L1/B7-H4 classifier genes

GO_ID	Description	p.adjust	qvalue
GO:0050663	cytokine secretion	0.001442	0.001089
GO:0009597	detection of virus	0.001442	0.001089
GO:0071346	cellular response to interferon-gamma	0.001864	0.001408
GO:0034341	response to interferon-gamma	0.003177	0.0024
GO:0045088	regulation of innate immune response	0.004586	0.003465
GO:0042110	T cell activation	0.007724	0.005836
GO:0050707	regulation of cytokine secretion	0.007724	0.005836
GO:0050715	positive regulation of cytokine secretion	0.007724	0.005836
GO:0032496	response to lipopolysaccharide	0.009715	0.00734
GO:0001819	positive regulation of cytokine production	0.009715	0.00734
GO:0002237	response to molecule of bacterial origin	0.012848	0.009706
GO:0034343	type III interferon production	0.012864	0.009718
GO:0034344	regulation of type III interferon production	0.012864	0.009718
GO:0032648	regulation of interferon-beta production	0.012864	0.009718
GO:0060333	interferon-gamma-mediated signaling pathway	0.013372	0.010102
GO:0032608	interferon-beta production	0.013372	0.010102
GO:0034138	toll-like receptor 3 signaling pathway	0.015171	0.011461
GO:0060760	positive regulation of response to cytokine stimulus	0.015171	0.011461
GO:0051607	defense response to virus	0.016976	0.012825
GO:0050717	positive regulation of interleukin-1 alpha secretion	0.017566	0.01327
GO:0032609	interferon-gamma production	0.018413	0.01391
GO:0051882	mitochondrial depolarization	0.018968	0.01433
GO:0050714	positive regulation of protein secretion	0.018968	0.01433
GO:0071222	cellular response to lipopolysaccharide	0.018968	0.01433
GO:0021778	oligodendrocyte cell fate specification	0.018968	0.01433
GO:0021779	oligodendrocyte cell fate commitment	0.018968	0.01433
GO:0021780	glial cell fate specification	0.018968	0.01433
GO:0050705	regulation of interleukin-1 alpha secretion	0.018968	0.01433
GO:0051209	release of sequestered calcium ion into cytosol	0.018968	0.01433
GO:0051283	negative regulation of sequestering of calcium ion	0.018968	0.01433
GO:0051282	regulation of sequestering of calcium ion	0.018968	0.01433
GO:0002683	negative regulation of immune system process	0.018968	0.01433
GO:0032647	regulation of interferon-alpha production	0.018968	0.01433
GO:0060759	regulation of response to cytokine stimulus	0.018968	0.01433
GO:0051208	sequestering of calcium ion	0.018968	0.01433
GO:0071219	cellular response to molecule of bacterial origin	0.018968	0.01433
GO:0032607	interferon-alpha production	0.018968	0.01433
GO:0002793	positive regulation of peptide secretion	0.018968	0.01433
GO:0032611	interleukin-1 beta production	0.018968	0.01433
GO:0042531	positive regulation of tyrosine phosphorylation of STAT protein	0.018968	0.01433

GO:2000108	positive regulation of leukocyte apoptotic process	0.018968	0.01433
GO:0032730	positive regulation of interleukin-1 alpha production	0.018968	0.01433
GO:0045349	interferon-alpha biosynthetic process	0.018968	0.01433
GO:0045354	regulation of interferon-alpha biosynthetic process	0.018968	0.01433
GO:0050703	interleukin-1 alpha secretion	0.018968	0.01433
GO:0072602	interleukin-4 secretion	0.018968	0.01433
GO:0032945	negative regulation of mononuclear cell proliferation	0.019307	0.014586
GO:0050672	negative regulation of lymphocyte proliferation	0.019307	0.014586
GO:0045089	positive regulation of innate immune response	0.019307	0.014586
GO:0032728	positive regulation of interferon-beta production	0.019307	0.014586

Table S9. Association between PD-L1/B7-H4 modules and clinicopathologic variables in TCGA grade2 gliomas (n=248)

Characteristics	**High-PDL1**	**High-B7H4**	**Double-Low**	**P**
Sex				
Male	30	33	72	0.846
Female	22	30	61	
NA				
Age (Years)				
＜40	24	33	81	0.160
≥40	28	30	52	
NA				
Extent of resection				
CR	33	32	81	0.408
ICR	15	28	42	
Biopsy	1	1	4	
NA				
Histology				
Oligodendroglioma	19	36	56	**0.013**
Astrocytoma	21	13	29	
Oligoastrocytoma	12	14	48	
NA				
TCGA subtype				
Mesenchymal	6	0	0	**< 0.0001**
Classical	3	0	0	
Neural	26	0	39	
Proneural	1	55	62	
NA				
IDH status				
Mutation	31	62	132	**< 0.0001**
Wild-type	20	0	1	
NA				
TERT status				
Mutation	11	16	32	0.400
Wild-type	24	22	39	
NA				
Chr1p/19q status				
Codeletion	5	29	59	**< 0.0001**
Non-codeletion	47	34	74	
NA				
MGMT Promoter status				
Unmethylated	22	3	10	**< 0.0001**
Methylated	30	60	132	

NA

Table S10. Association between PD-L1/B7-H4 modules and clinicopathologic variables in TCGA grade3 gliomas (n=262)

Characteristics	High-PDL1	High-B7H4	Double-Low	P
Sex				
Male	35	52	60	0.162
Female	37	43	35	
NA				
Age (Years)				
< 40	11	45	46	**< 0.0001**
≥40	61	50	49	
NA				
Extent of resection				
CR	36	55	61	0.114
ICR	34	36	30	
Biopsy	1	0	0	
NA				
Histology				
Oligodendroglioma	9	32	38	**< 0.0001**
Astrocytoma	53	43	33	
Oligoastrocytoma	10	20	24	
NA				
TCGA subtype				
Mesenchymal	21	0	3	**< 0.0001**
Classical	30	0	4	
Neural	3	5	31	
Proneural	0	75	25	
NA				
IDH status				
Mutation	15	93	80	**< 0.0001**
Wild-type	57	2	14	
NA				
TERT status				
Mutation	30	18	23	**0.001**
Wild-type	11	32	30	
NA				
Chr1p/19q status				
Codeletion	2	37	36	**< 0.0001**
Non-codeletion	70	58	59	
NA				
MGMT Promoter status				
Unmethylated	31	5	18	**< 0.0001**

Methylated	41	90	77	
NA				

Table S11. Association between PD-L1/B7-H4 modules and clinicopathologic variables in TCGA grade4 gliomas (n=156)

Characteristics	**High-PDL1**	**High-B7H4**	**Double-Low**	**P**
Sex				
Male	22	24	55	0.804
Female	12	10	32	
NA				
Age (Years)				
＜40	2	7	3	**0.01**
≥40	32	27	84	
NA				
TCGA subtype				
Mesenchymal	30	3	34	**< 0.0001**
Classical	1	4	44	
Neural	3	0	3	
Proneural	0	18	0	
NA				
IDH status				
Mutation	0	10	1	**< 0.0001**
Wild-type	33	23	85	
NA				
TERT status				
Mutation	3	5	17	**0.011**
Wild-type	0	4	0	
NA				
MGMT Promoter status				
Unmethylated	19	13	39	0.105
Methylated	6	13	33	
NA				

Table S12. Association between PD-L1/B7-H4 modules and clinicopathologic variables in CGGA grade2 gliomas (n=109)

Characteristics	**High-PDL1**	**High-B7H4**	**Double-Low**	**P**
Sex				
Male	15	18	32	0.256
Female	8	19	17	
Age (Years)				
＜40	11	26	21	**0.036**
≥40	12	11	18	
Extent of resection				
CR	3	8	14	0.932
ICR	4	14	19	
NA				
Histology				
Oligodendroglioma	2	8	17	**0.001**
Astrocytoma	17	17	10	
Oligoastrocytoma	4	12	22	
TCGA subtype				
Mesenchymal	7	0	0	**＜0.0001**
Classical	7	0	2	
Neural	6	6	42	
Proneural	3	31	5	
IDH status				
Mutation	15	37	42	**＜0.0001**
Wild-type	8	0	7	
Chr1p/19q status				
Codeletion	3	13	23	**0.02**
Non-codeletion	19	24	25	
NA				

Table S13. Association between PD-L1/B7-H4 modules and clinicopathologic variables in CGGA grade3 gliomas (n=72)

Characteristics	High-PDL1	High-B7H4	Double-Low	P
Sex				
Male	8	16	21	0.612
Female	4	7	16	
Age (Years)				
＜40	1	13	18	**0.019**
≥40	11	10	19	
Extent of resection				
CR	5	9	14	0.683
ICR	2	8	13	
NA				
Histology				
Oligodendroglioma	1	7	5	0.087
Astrocytoma	6	3	15	
Oligoastrocytoma	5	13	17	
TCGA subtype				
Mesenchymal	9	0	1	**< 0.0001**
Classical	3	0	14	
Neural	0	1	14	
Proneural	0	22	8	
IDH status				
Mutation	0	21	16	**< 0.0001**
Wild-type	12	2	21	
Chr1p/19q status				
Codeletion	0	6	5	0.113
Non-codeletion	12	17	32	
NA				

Table S14. Association between PD-L1/B7-H4 modules and clinicopathologic variables in CGGA grade4 gliomas (n=144)

Characteristics	High-PD-L1	High-B7-H4	Double-Low	P
Sex				
Male	38	27	28	0.51
Female	16	16	19	
Age (Years)				
＜40	6	21	8	**< 0.0001**
≥40	48	22	39	
Extent of resection				
CR	20	6	17	0.614
ICR	16	5	8	
NA				
TCGA subtype				
Mesenchymal	40	1	10	**< 0.0001**
Classical	10	7	31	
Neural	4	2	6	
Proneural	0	33	0	
IDH status				
Mutation	0	34	2	**< 0.0001**
Wild-type	54	9	45	
Chr1p/19q status				
Codeletion	1	3	1	0.38
Non-codeletion	48	33	40	
NA				