Inhibition of TAZ contributes radiation-induced senescence and growth arrest in glioma cells

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Supplemental materials

Figure S1 Radiation induces cellular senescence in LN229 cells. (a) Cells were treated with indicated doses of ionizing radiation and subjected to colony formation assay. N=3. (b) Cells were treated with or without 8 Gy of ionizing radiation, cultured for 7 days, and subjected to β-gal staining. A field in each of the left panel was enlarged and shown in the correspondent right panel. Scale bars=200 μM. (c) Cells were treated with or without 8 Gy of ionizing radiation, cultured for indicated days, and subjected to β-gal staining. N=3. **P<0.01, ***P<0.001. (d) and (e) Cells were treated with or without 8 Gy of ionizing radiation, cultured for indicated days, and subjected to p-H2AX staining. Scale bars=20 μM. Representative images from two independent experiments were shown.

Figure S2 Gene expression in GBM cell lines. (a) A panel of indicated GBM cell lines cultured in regular petri dish were lysed and analyzed by western blotting. Representative blots from two independent experiments were shown. (b) and (c) the indicated GBM cells were cultured as in (a) and subjected to q-RT-PCR. N=2.

Table S1. Clinical information of tumor samples

Patient No.	Gender	Age	Diagnosis	WHO Grade	Location	Treatment	PFS (months)	OS (months)
1	F	60	glioblastoma	4	left frontal lobe	TMZ(1)		3.5
2	M	38	high grade glioma(WHO III),mainly are presented as oligodendroglioma	3	right frontal lobe	RT+TMZ(6)		20
3	M	58	anaplastic astrocytoma(WHO III)	3	right temporal lobe	RT+TMZ(1)		28
4	M	48	diffuse astrocytoma(WHO II)	2	righe frontal lobe	RT+TMZ(6)		33
5	F	44	oligoastrocytoma(WHO II)	2	right temporal lobe	RT+TMZ(6)		36
6	M	52	diffuse astrocytoma(WHO II)	2	right temporal and insular lobe	RT+TMZ(6)		32
7	M	56	glioblastoma	4	right temporal lobe	first:RT+TMZ(6)； recurrence： surgery+BEV+CPT-11		32
8	M	56	glioblastoma	4	right temporal lobe	first:RT+TMZ(6) +IT; recurrence:surgery+TMZ(6)+BEV		24
9	M	33	glioblastoma	4	left occipital lobe	first:none; recurrence:surgery+RT+TMZ(6)		24
10	F	55	glioblastoma	4	left frontoparietal lobe	first:RT+TMZ(6); recurrence:surgery+TMZ		26
11	F	44	glioblastoma	4	left frontoparietal lobe	TMZ(2)		26
12	M	49	glioblastoma	4	right temporal lobe	TMZ(6)		18
13	F	5	glioblastoma	4	right frontal lobe	none		5
14	M	65	glioblastoma	4	right frontal lobe	RT		19
15	F	60	glioblastoma	4	left frontal lobe	TMZ(1)		3.5
16	M	38	glioblastoma	4	right frontal lobe	RT+TMZ(6)		20
17	M	58	glioblastoma	4	right temporal lobe	RT+TMZ(6)		28
18	M	61	glioblastoma	4	right temporal lobe	TMZ(2)		3
19	M	47	glioblastoma	4	right frontotemporal lobe and basal ganglia area	RT+TMZ(4)		12
20	F	72	glioblastoma	4	right frontotemporal and insular lobe	TMZ(1)		2
21	M	44	glioblastoma	4	right frontal lobe	TMZ(6)		15
22	F	73	glioblastoma	4	left temporal lobe	RT+TMZ（6）+BEV		32
23	F	50	glioblastoma	4	lefe parietal lobe	RT+TMZ（6）		40
24	F	49	glioblastoma	4	right frontal lobe	TMZ（6）		9
25	M	55	gliosarcoma	4	right frontal lobe	RT+TMZ（6）		12
26	M	63	glioblastoma	4	left temporal lobe	TMZ（3）		6
27	M	58	glioblastoma	4	right temporal and insular lobe, bilateral basal ganglia area	none		7
28	M	35	glioblastoma	4	left occipitotemporal lobe	TMZ(6)		8
29	M	30	glioblastoma	4	left frontal lobe	RT+TMZ(6)		40
30	F	56	glioblastoma	4	left frontotemporal lobe	RT+TMZ(6)		18
31	F	31	glioblastoma	4	right frontal lobe	chinese medicine		13
32	M	64	glioblastoma	4	left temporoparietal lobe	RT+TMZ(6)		20
33	M	55	glioblastoma	4	left temporal lobe	TMZ(1)		2.5
34	F	56	recurrent glioblastoma	4	left frontal lobe	RT+TMZ(6)	2	8
35	M	58	recurrent glioblastoma	4	right occipitotemporal lobe	RT+TMZ(6)	8	10

36	M	57	recurrent glioblastoma	4	right frontotemporal lobe	RT+TMZ(?)+IT	11	12
37	M	34	recurrent glioblastoma	4	left occipital lobe	RT+TMZ	8	12
38	F	56	recurrent glioblastoma	4	left frontoparietal lobe	TMZ	12	13
39	F	46	recurrent glioblastoma	4	right frontal lobe	first:RT+TMZ(2); recurrence:surgery+TMZ	24	17
40	M	49	recurrent glioblastoma	4	right temporal lobe	TMZ	18	9
41	F	53	recurrent glioblastoma	4	left frontal lobe	TMZ	36	12
42	F	50	recurrent glioblastoma	4	left occipitotemporal lobe	TMZ	13	15
43	M	37	recurrent glioblastoma	4	left frontal lobe	first:RT+TMZ（6）; recurrence:surgerry+TMZ	8	6
44	F	52	recurrent glioblastoma	4	right frontal lobe	first:AED; recurrence:RT+TMZ(6)	27	40
45	M	32	recurrent anaplastic astrocytoma(WHO III)	3	right parietal lobe	RT+TMZ(6)	20	8
46	M	36	Recurrent anaplastic astrocytoma(WHO III)	3	left frontoparietal lobe	RT+TMZ(6)	13	23
47	M	53	Recurrent glioblastoma	4	left temporal lobe	RT+CPT-11+DDP	13	24
48	F	58	recurrent anaplastic astrocytoma(WHO III)	3	right occipitoparietal lobe	RT+TMZ(6)	4	14
49	F	47	recurrent anaplastic astrocytoma(WHO III)	3	left frontal lobe	RT+TMZ(18)	4	28
50	F	54	recurrent glioblastoma	4	right parietal lobe	none	14	25
51	M	33	recurrent glioblastoma	4	right frontal lobe	first:RT+TMZ(4)+chinese medicine; recurrence:surgery+TMZ+CPT-11+BEV(6)	5	10
52	F	44	recurrent glioblastoma	4	right frontal lobe	TMZ(6)	10	38
53	F	51	recurrent glioblastoma	4	right frontal lobe	none	25	2
54	F	55	recurrent anaplastic astrocytoma(WHO III)	3	left parietal lobe	first:RT+TMZ(6) recurrence:TMZ(6)	5	18
55	F	6	recurrent glioblastoma	4	right frontotemporal lobe	none	5	5
56	M	65	recurrent glioblastoma	4	left frontoparietal lobe	RT	3	15
57	M	60	recurrent glioblastoma	4	left parietoccipital lobe	RT+TMZ(6)	5	8
58	F	43	recurrent anaplastic astrocytoma(WHO III)	3	right temporal lobe	RT	15	10
59	F	40	recurrent anaplastic astrocytoma(WHO III)	3	right frontal lobe	first:chinese medicine; +TMZ	12	30
60	F	38	recurrent glioblastoma	4	right temporal lobe	none	10	15
61	M	42	recurrent glioblastoma	4	right temporal lobe	chinese medicine	5	15

Table S2. Cellular distribution of TAZ in GBM tumors.

	PT	Primary	IR-R	No-IR-R
Cytoplasm (%)	100	44	94	13
Cytoplasm/Nucleus (%)	0	56	6	87

Cytoplasm: only cytoplasmic compartment show TAZ signal.
Cytoplasm/Nucleus: both cytoplasmic and nuclear compartments show TAZ signal.

Table S3. Cellular distribution of β-catenin in GBM tumors.

	PT	Primary	IR-R	No-IR-R
Cytoplasm (%)	100	71	71	37
Cytoplasm/Nucleus (%)	0	29	29	63

Cytoplasm: only cytoplasmic compartment show β-catenin signal.
Cytoplasm/Nucleus: both cytoplasmic and nuclear compartments show β-catenin signal.

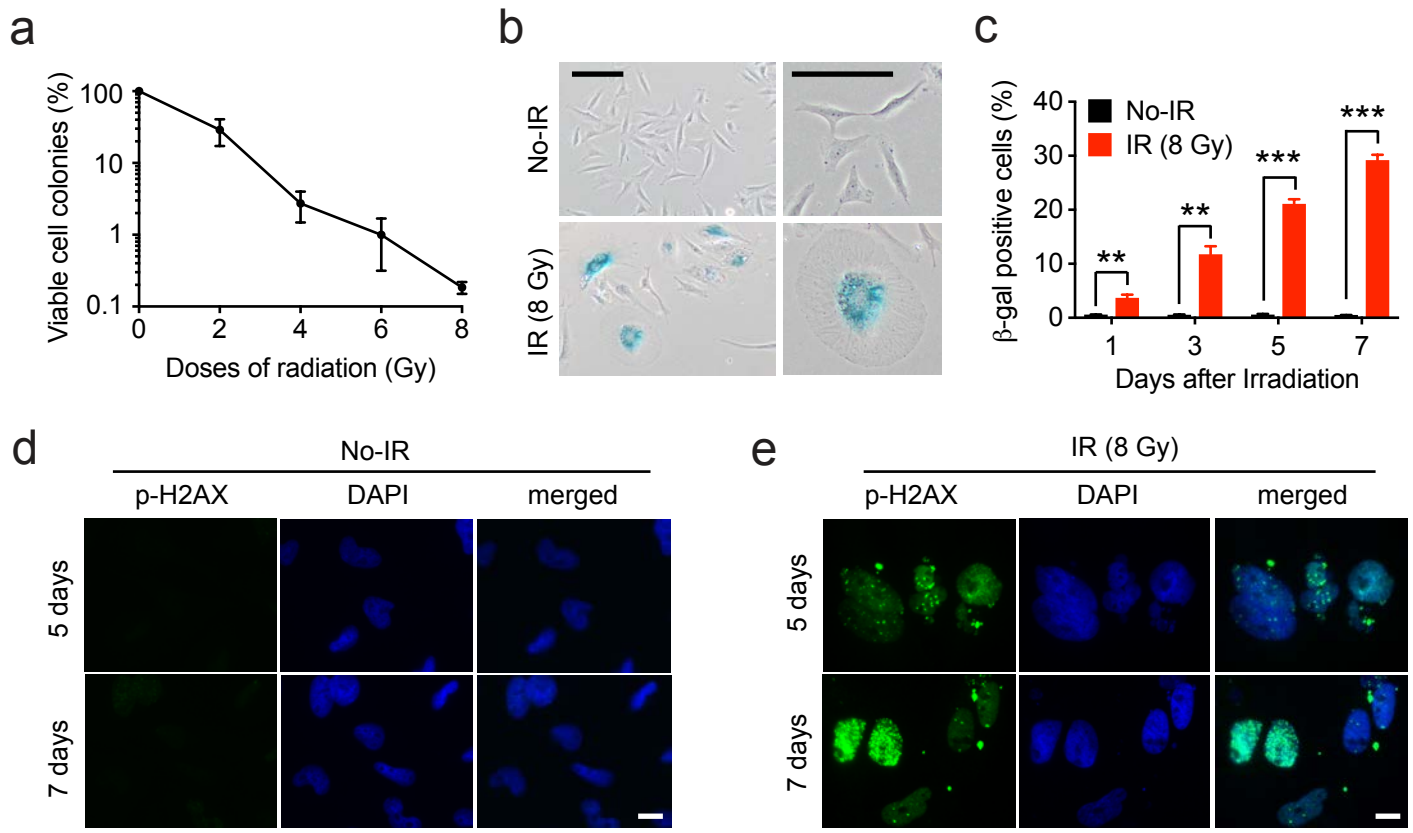
a
Viable cell colonies (%)
100
10
1
0.1
0
2
4
6
8
Doses of radiation (Gy)
b
No-IR
IR (8 Gy)
c
β-gal positive cells (%)
40
30
20
10
0
No-IR
IR (8 Gy)
**
**
***
***
1
3
5
7
Days after Irradiation
d
No-IR
p-H2AX
DAPI
merged
5 days
7 days
e
IR (8 Gy)
p-H2AX
DAPI
merged
5 days
7 days

Figure S1

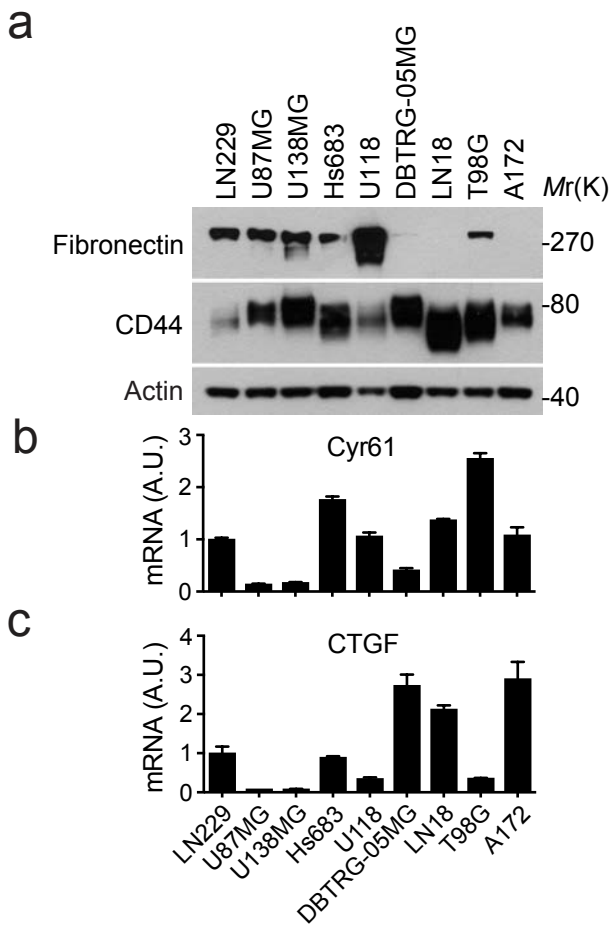
a
LN229
U87MG
U138MG
Hs683
U118
DBTRG-05MG
LN18
T98G
A172
Mr(K)
Fibronectin
CD44
Actin
270
80
40
b
Cyr61
mRNA (A.U.)
0
1
2
3
c
CTGF
mRNA (A.U.)
0
1
2
3
4
LN229
U87MG
U138MG
Hs683
U118
DBTRG-05MG
LN18
T98G
A172

Figure S2