HOKKAIDO UNIVERSITY

Title	Toll-like receptor 3 signal augments radiation-induced tumor growth retardation in a murine model
Author(s)	Yoshida, Sumito; Shime, Hiroaki; Takeda, Yohei et al.
Citation	Cancer science, 109(4), 956-965 https://doi.org/10.1111/cas.13543
Issue Date	2018-04
Doc URL	https://hdl.handle.net/2115/70877
Rights(URL)	https://creativecommons.org/licenses/by-nc/4.0/
Type	journal article
File Information	cas13543-sup-0001-supinfo.pdf, Supporting Information

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Table S1. Antiboides used in this study.

Antibody (and related reagent)	Clone	Supplier
FITC-anti-mouse CD3ε	145-2C11	Biolegend
PE/Cy7-anti-mouse CD3	17A2	
AlexaFluor700-anti-mouse CD8α APC-anti-mouse CD8α	53-6.7	
PE/Cy7-anti-mouse/human CD11b	M1/70	
APC-anti-mouse CD11c PE/Cy7-anti-mouse CD11c	N418	
APC-anti-mouse/human CD44	IM7	
AlexaFluor700-anti-mouse CD45.2	104	
APC/Cy7-anti-mouse CD45.2	30-F11	
FITC-anti-mouse CD62L	MEL-14	
APC-anti-mouse F4/80 FITC-anti-mouse F4/80	BM8	
APC-anti-mouse Ly6G/Ly6C (Gr-1)	RB6-8C5	
PE-anti-mouse TNF-α	MP6-XT22	
Purified anti-mouse CD16/32 *used in Fc blocking	93	
PE-Rat IgG1, κ *Isotype control of PE-anti-mouse TNF-α	BM2a	eBioscience
T-select H-2Kb OVA Tetramer-SIINFEKL-PE		MBL
ViaProbe *used for excluding dead cells		BD Bioscience

Table S2. Primers for RT-qPCR used in this study.

	Forward	Reverse
Ccl3	5'-TTGAAACCAGCAGCCTTTGC-3'	5'-CTTTGGAGTCAGCGCAGATCT-3'
Ccl4	5'-GCCCTCTCTCTCCTCTTGCT-3'	5'-GGAGGGTCAGAGCCCATT-3'
Ccl5	5'-TGCCCACGTCAAGGAGTATTT-3'	5'-TCGAGTGACAAACACGACTGC-3'
Cxcl9	5'-GATAAGGAATGCACGATGCTC-3'	5'-TCTCCGTTCTTCAGTGTAGCAA-3'
Cxcl10	5'-GTGTTGAGATCATTGCCACGA-3'	5'-GCGTGGCTTCACTCCAGTTAA-3'
Cxcl11	5'-GGCTGCGACAAAGTTGAAGTGA-3'	5'-TCCTGGCACAGAGTTCTTATTGGAG-3'
Fasl	5'-TTTCAGAGGGTGTACTGGGG-3'	5'-TGGTTGGAATGGGATTAGGA-3'
Gapdh	5'-GCCTGGAGAAACCTGCCA-3'	5'-CCCTCAGATGCCTGCTTCA-3'
Gzmb	5'-GCCTGGAGAAACCTGCCA-3'	5'-CCCTCAGATGCCTGCTTCA-3'
Ifnb	5'-CCAGCTCCAAGAAAGGACGA-3'	5'-CGCCCTGTAGGTGAGGTTGAT-3'
Ifng	5'-GATATCTGGAGGAACTGGCAAAAG-3'	5'-AGAGATAATCTGGCTCTGCAGGAT-3'
Prf1	5'-CAAGGTAGCCAATTTTGCAGC-3'	5'-GGCGAAAACTGTACATGCGAC-3'
Tbx21	5'-CAACCAGCACCAGACAGAGA-3'	5'-CCACATCCACAAACATCCTG-3'

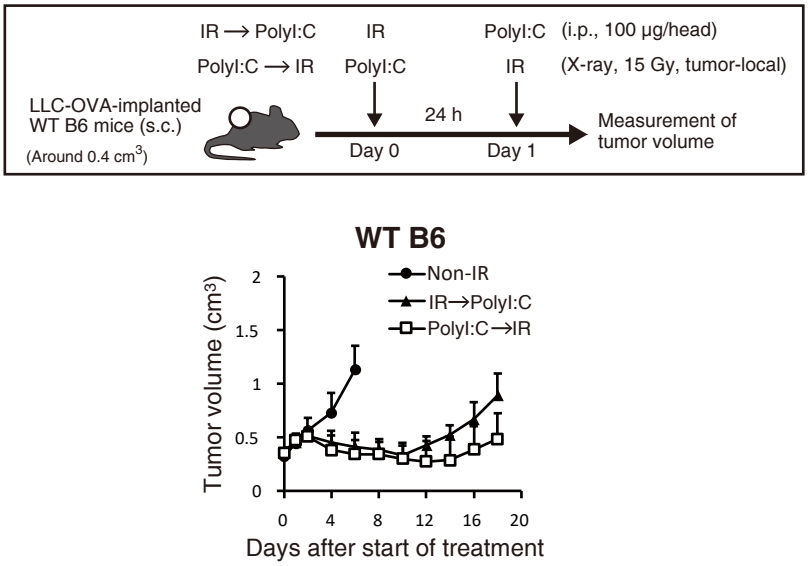


Figure S1. Pre-treatment of polyI:C with IR was more efficient than post-treatment in tumor regression. LLC-OVA-implanted WT B6 mice were intraperitoneally injected with polyI:C (100 μg/head) or performed 15 Gy of ionizing radiation (IR) when tumor volume reached around 0.4 cm^3. After 24 h, polyI:C administration or IR was performed following the time table in this figure. *n* = 3-4 mice per group. Data are shown as average (SD) tumor size.

Yoshida et.al.

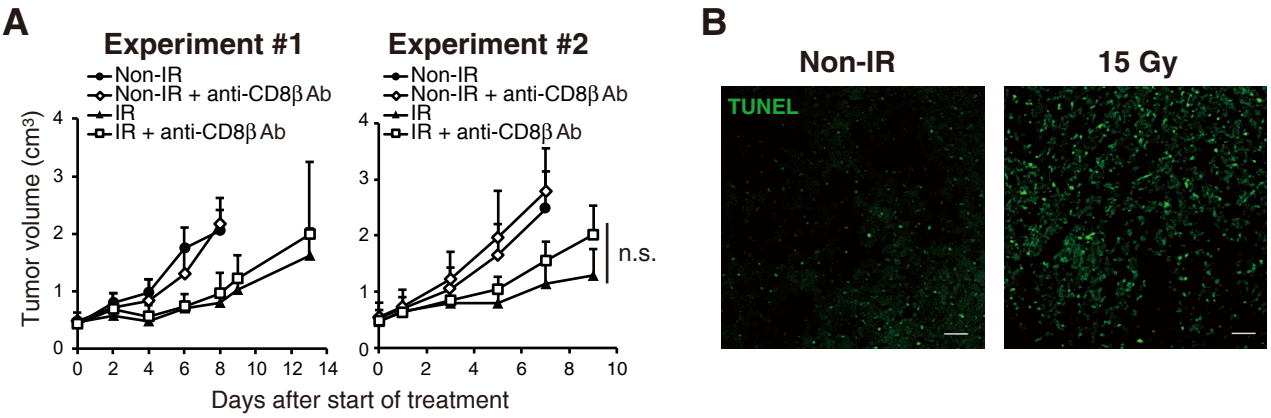


Figure S2. IR damaged tumor cells and suppressed tumor growth independent of CTLs. (A) Growth of LLC-OVA tumor on WT B6 mice with or without anti-CD8β antibody (Ab) treatment. Anti-CD8β Ab was injected into tumor-bearing mice 24-48 h before 15 Gy of ionizing radiation (IR). Additional Ab injection was performed on 5-6 days after irradiation. Similar two experiments were performed. Data are shown as average (SD) tumor size. n.s., not significant. In experiment #1, n = 3 mice per group. In experiment #2, n = 3-5 mice per group. (B) LLC-OVA-implanted mice were locally irradiated with 15 Gy of X-ray on tumor site. After 48 h, tumors were harvested, fixed with 10% neutralized buffered formalin at 4 °C for 3 h. Then, samples were equilibrated with 15% and 30% sucrose/PBS, mounted in O.C.T. compound (Sakura Finetek Japan), and flozen. Blocks were sliced and stained with the In Situ Cell Death Detection Kit, Fluorescein (Roche). Specimens were analyzed using BZ-9000 (KEYENCE). Scale bars, 50 μm. The pictures are representative of two independent experiments.

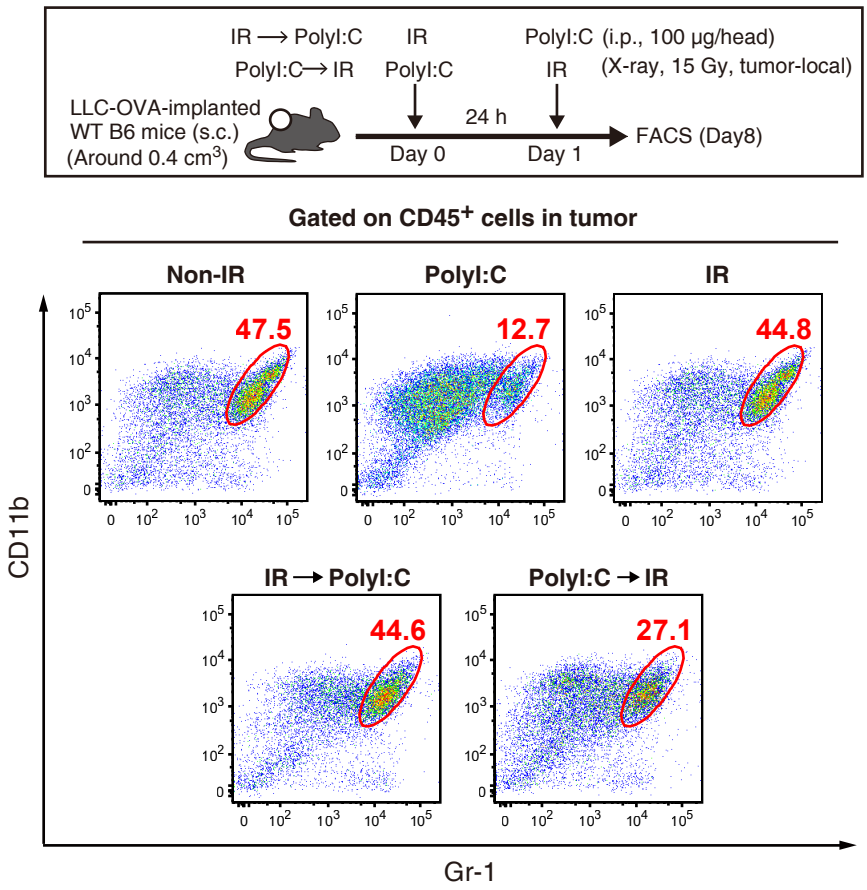


Figure S3. Frequency of intratumor CD11b$^+$ Gr-1$^+$ cells was different in pre- or post- polyI:C injection with radiation. LLC-OVA-implanted WT B6 mice were treated with polyI:C and IR following the time table in this figure. Tumors were harvested on day 8 after the start of treatment and FACS analysis was performed. Plots are representative results of two with similar outcomes.

Yoshida et.al.

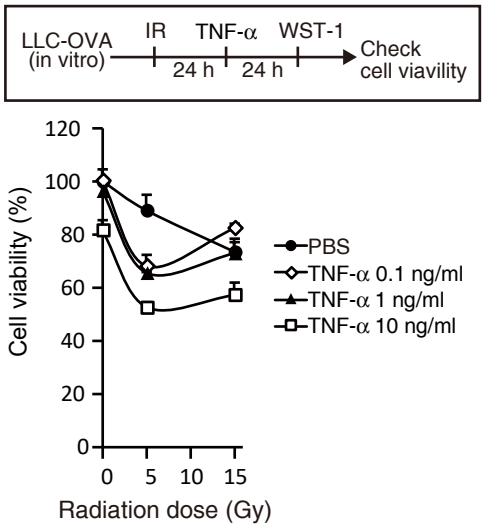


Figure S4. TNF-α post-treatment decreased LLC-OVA cell viability combinatry with IR. LLC-OVA cells were subjected to 15 Gy of IR and 24 h later, TNF-α was treated . After 24 h culture, cell viability was assessed by WST-1 assay. *n* = 3. Data represent the means (SD).