sciendo Radiology and Oncology | Ljubljana | Slovenia | www.radioloncol.com RADIOLOGY AND ONCOLOGY

Co-treatment with vactosertib, a novel, orally bioavailable activin receptor-like kinase 5 inhibitor, suppresses radiotherapy-induced epithelial-to-mesenchymal transition, cancer cell stemness, and lung metastasis of breast cancer

Jiwon Choi, Jiyoung Park, Ilyoung Cho, Yhunyhong Sheen

doi: 10.2478/raon-2022-0012

Supplementary Tables

A.1. Antibodies for western blot analysis

A.1.1. Primary antibodies

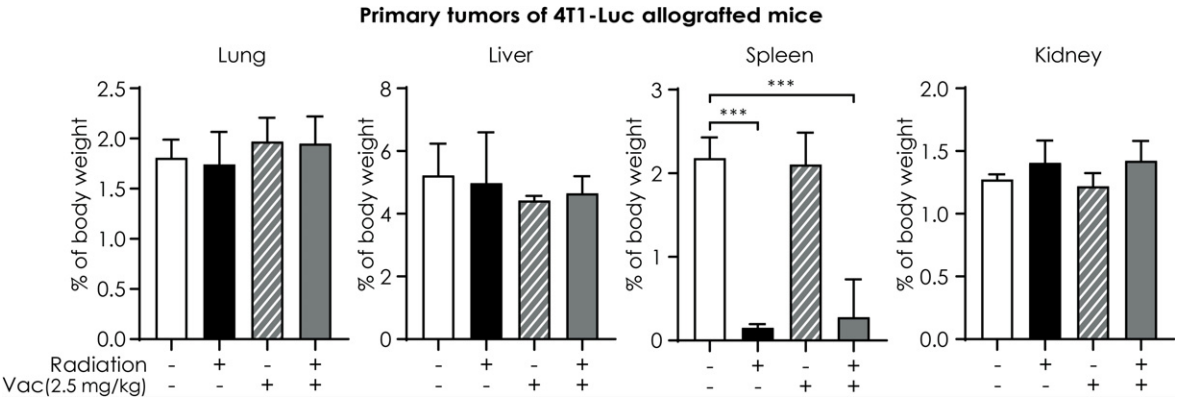
Target	Company	Product number
p-SMAD2/3	Santa Cruz Biotechnology, Santa Cruz, CA, USA	sc-11769
SMAD2/3	BD Biosciences, San Diego, CA, USA	610842
SNAIL	Cell Signaling, Beverly, MA, USA	3879
SLUG	Cell Signaling, Beverly, MA, USA	9585
TWIST	Santa Cruz Biotechnology, Santa Cruz, CA, USA	sc-15393
VIMENTIN	Santa Cruz Biotechnology, Santa Cruz, CA, USA	sc-32322
FIBRONECTIN	BD Biosciences, San Diego, CA, USA	610077
E-CADHERIN	BD Biosciences, San Diego, CA, USA	610182
N-CADHERIN	BD Biosciences, San Diego, CA, USA	610920
NOX4	Santa Cruz Biotechnology, Santa Cruz, CA, USA	sc-30141
4HNE	Santa Cruz Biotechnology, Santa Cruz, CA, USA	ab46545
NANOG	Abcam, Cambridge, MA, USA	ab109250
OCT4	Abcam, Cambridge, MA, USA	ab109183
SOX2	Abcam, Cambridge, MA, USA	ab92494
C-MYC	Santa Cruz Biotechnology, Santa Cruz, CA, USA	ab32072
KLF4	Abcam, Cambridge, MA, USA	ab151733
SCA1	Abcam, Cambridge, MA, USA	ab109211
GAPDH	Sigma Aldrich, St. Louis, MO, USA	MAB374
β-ACTIN	Sigma Aldrich, St. Louis, MO, USA	A5316

A.1.2 Secondary antibodies

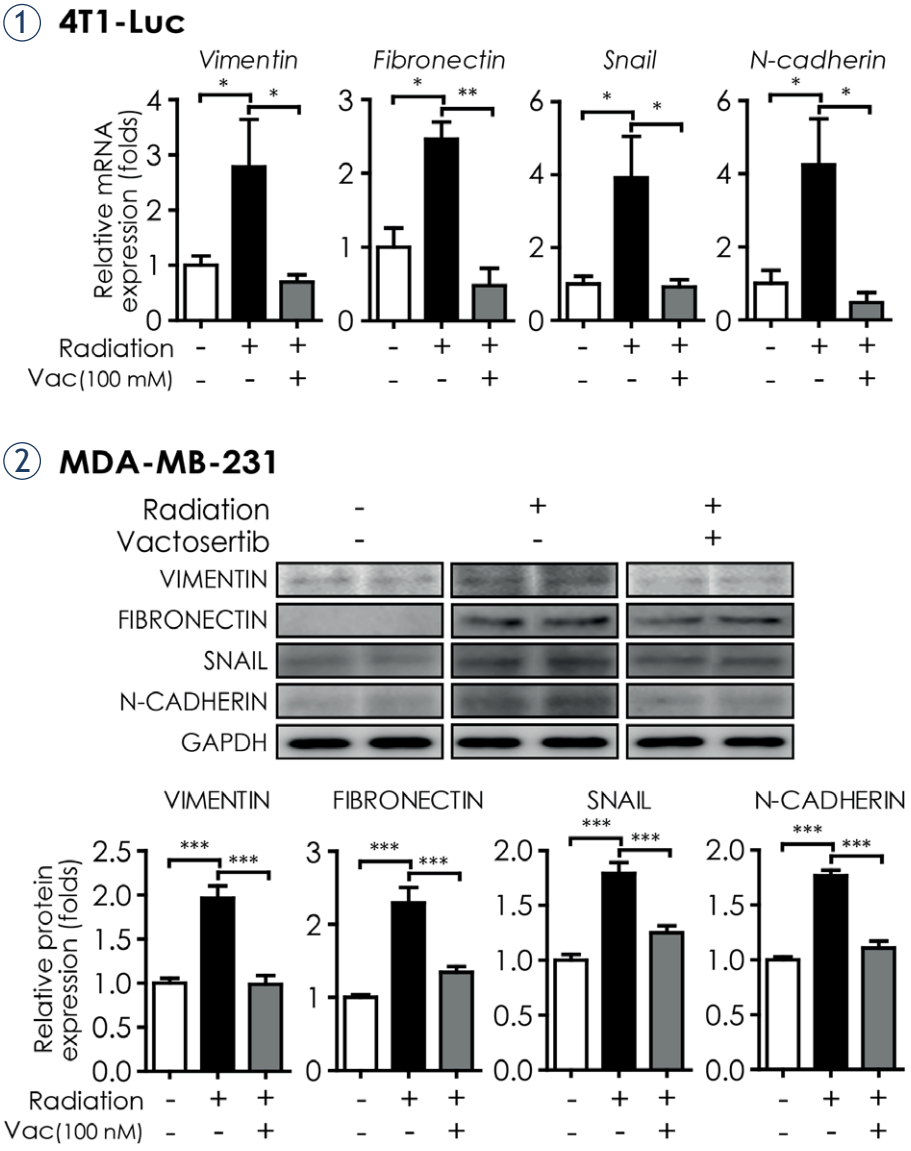
Antibody	Company	Product number
Anti-rabbit HRP	GenDEPOT, Katy, TX, USA	SA002-500
Anti-mouse HRP	GenDEPOT, Katy, TX, USA	SA001-500

A.2. Primers for qRT-PCR

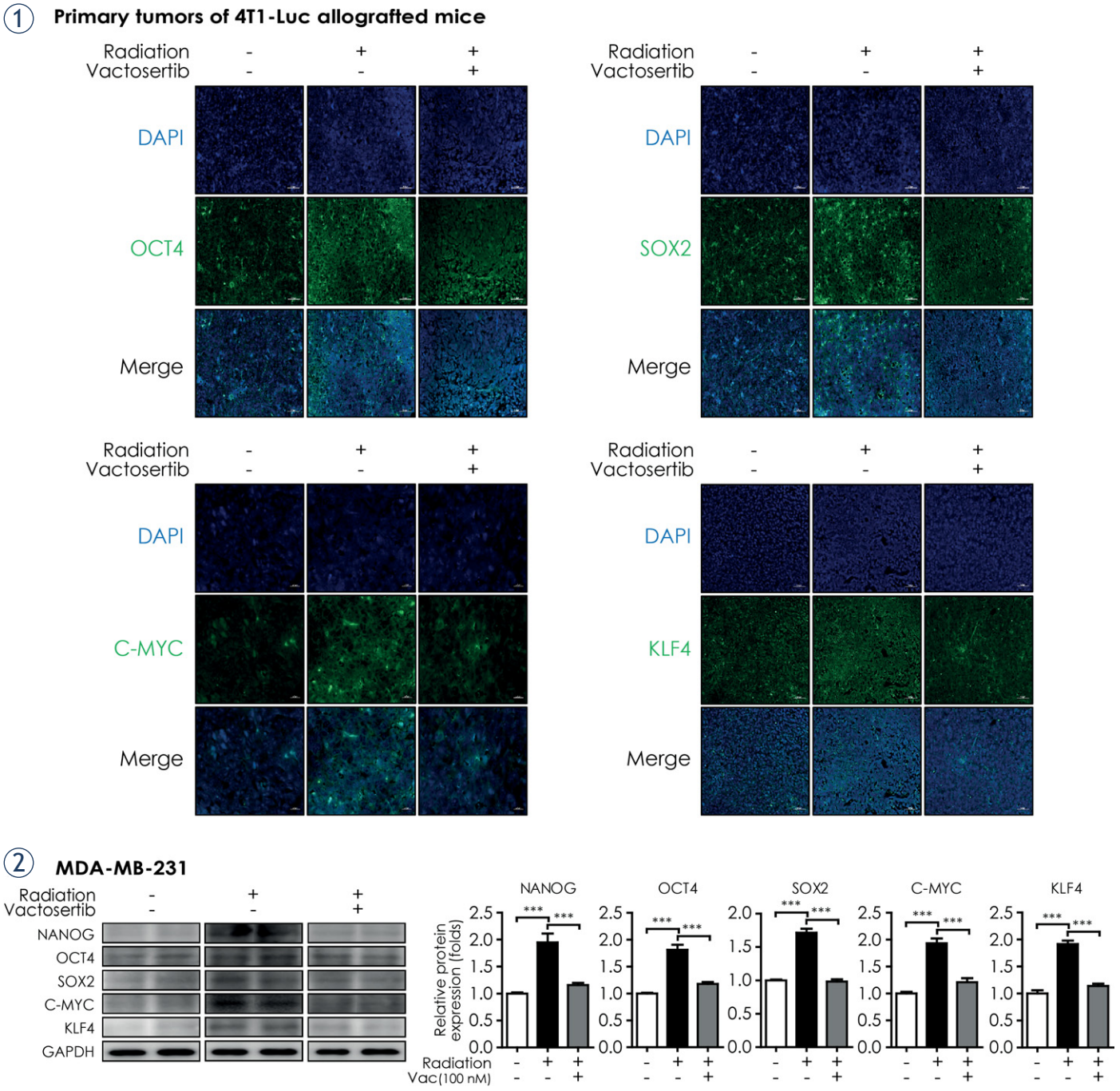
Gene	Forward (5' – 3')	Reverse (5' – 3')
Vimentin	ACCCGCACCAACGAGAAGGT	ATTCTGCTGCTCCAGGAAGCG
Fibronectin	GCTTCAAGCTGGGTGTACGA	AAGTTGGTTGGGGGAGACAG
Snail	GACGCTGAAGTATCCGATAGAACACG	CCGGACATGGCCTTGTAGCA
N-cadherin	GTGCCATTAGCCAAGGGAAT	GCGTTCCTGTTCCACTCATA
Nox4	TCTGTTGTGGACCCAATTCA	AGCTGATTGATTCCGCTGAG
Nanog	TGGGATTTACAGGCGTGAGC	AAGCAAAGCCTCCCAATCCC
Oct4	GGGCTCTCCCATGCATTCAA	CACCTTCCCTCCAACCAGTT
Sox2	TCGGCAGACTGATTCAAATAATACAG	CCATGCAGGTTGACACCGTTG
c-Myc	GCGTCCTGGGAAGGGAGATCCGGAGC	TTGAGGGGCATCGTCGCGGGAGGCTG
Klf4	ACGATCGTGGCCCCGGAAAA	CAACAACCGAAAATGCACCAGCCCCA
Ppia	TGCACAGACGGTCACTCAAA	TGCCATCGCCAAGGAGTAG



SUPPLEMENTARY FIGURE A. Changes in organ weight by radiation and vactisertib. Organ weights in each group were calculated as relative organ weights to the weight of the mice. The spleen weight was significantly decreased in the radiotherapy group and in the radiotherapy and vactosertib group, but there was little change in weight in other organs.



SUPPLEMENTARY FIGURE B. (1) Relative mRNA expression of Vimentin, Fibronectin, Snail, and N-cadherin in 4T1-Luc cells. The mRNA expression level of each marker was normalized by that of Ppia mRNA. **(2)** Protein expressions of VIMENTIN, FIBRONECTIN, SNAIL, and N-CADHERIN in MDA-MB-231 cells were examined by western blot analysis. Protein expression was normalized by that of GAPDH.1.



SUPPLEMENTARY FIGURE C. (1) Fluorescence immunohistochemistry analysis of OCT4, SOX2, C-MYC and KLF4 in irradiated primary tumors of 4T1-Luc allografted BALB/c syngeneic mice. In confocal images, green fluorescence indicates OCT4, SOX2, C-MYC, and KLF4 (magnification: 20×, scale bar: 50 μm). Representative images are shown from three independent experiments. **(2)** Protein expression of pluripotent stem cell regulators (NANOG, OCT4, SOX2, C-MYC and KLF4) in MDA-MB-231 cells was determined by western blot analysis. Protein expression was normalized by that of GAPDH. Data represent means of three independent experiments performed in triplicate. Significance evaluation was performed by one-way analysis of variance (ANOVA) with Bonferroni post-hoc test (*, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.005$, respectively).