

Supplementary Information

Oncolytic virotherapy reverses chemoresistance in osteosarcoma by suppressing MDR1 expression

Kazuhisa Sugi¹, Hiroshi Tazawa^{2,3}, Joe Hasei¹, Yasuaki Yamakawa¹,
Toshinori Omori¹, Tadashi Komatsubara¹, Yusuke Mochizuki¹, Hiroya Kondo¹,
Shuhei Osaki¹, Tomohiro Fujiwara¹, Toshiyuki Kunisada^{1,4}, Aki Yoshida¹, Koji Ueda⁵,
Yasuo Urata⁶, Shunsuke Kagawa^{2,7}, Toshifumi Ozaki¹, and Toshiyoshi Fujiwara²

Departments of ¹Orthopaedic Surgery, ²Gastroenterological Surgery, and ⁴Medical Materials for Musculoskeletal Reconstruction, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan. ³Center for Innovative Clinical Medicine, and ⁷Minimally Invasive Therapy Center, Okayama University Hospital, Okayama 700-8558, Japan. ⁵Project for Personalized Cancer Medicine, Cancer Precision Medicine Center, Japanese Foundation for Cancer Research, Tokyo, 135-8550, Japan. ⁶Oncolys BioPharma, Inc., Tokyo 105-0001, Japan.

Supplementary Methods

Supplementary Fig. S1

Comparison of the proliferation of parental and DOX-resistant OS cells.

Supplementary Fig. S2

Comparison of the morphology of parental and DOX-resistant OS cells.

Supplementary Fig. S3

Comparison of the CAR expression on the surface of parental and DOX-resistant OS cells.

Supplementary Fig. S4

Tumor growth curve of individual mice bearing DOX-resistant MNNG/HOS tumors.

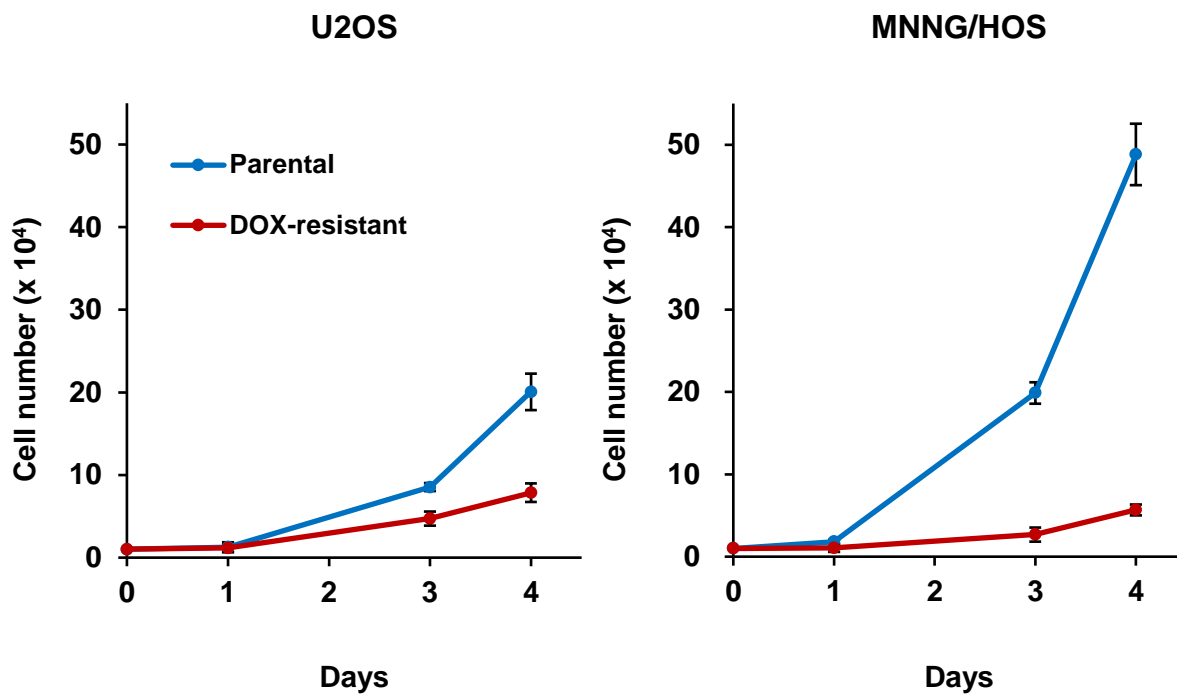
Supplementary Methods

Cell proliferation assay

Four human OS cell lines (U2OS, DOX-resistant U2OS, MNNG/HOS, DOX-resistant MNNG/HOS) were seeded at a density of 10^4 cells/well in 24-well tissue culture plates. Cells were counted on days 1, 3, and 4. The average number of cells was determined at each time point from triplicate samples. Cell morphology was examined under a light microscope.

Flow cytometric analysis

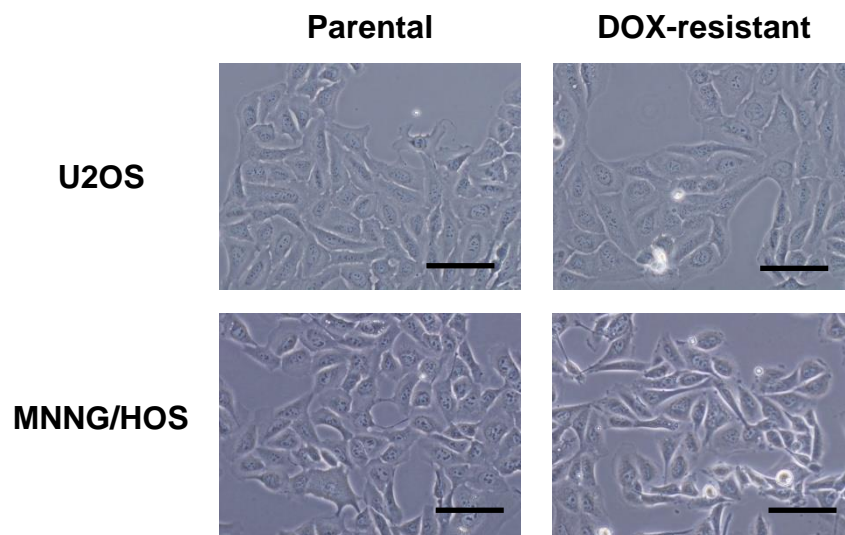
To analyze the expression of coxsackie and adenovirus receptor (CAR), cells were incubated with mouse anti-CAR primary antibody (Upstate Biotechnology, Lake Placid, NY, USA) or isotype control IgG for 60 min on ice. Then cells were labeled with fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG secondary antibody (Invitrogen, Carlsbad, CA, USA) for 30 min, and were then analyzed using flow cytometry (FACSLytic; BD Biosciences, San Jose, CA, USA). The mean fluorescence intensity (MFI) for each cell line was determined by calculating the difference between the MFI in antibody-treated and isotype control IgG-treated cells from three independent experiments.



Supplementary Fig. S1

Comparison of the proliferation of parental and DOX-resistant OS cells.

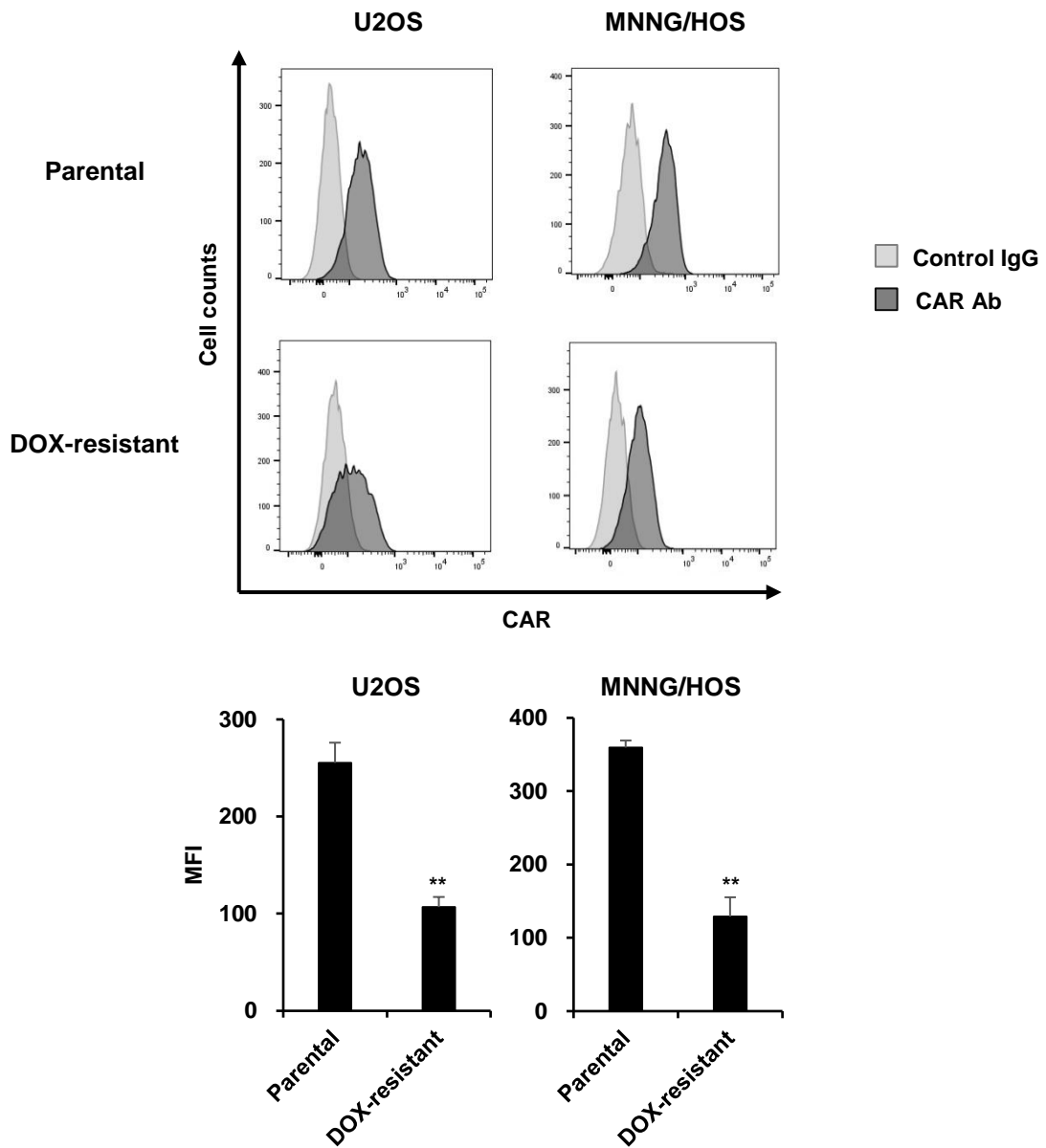
Parental and DOX-resistant U2OS and MNNG/HOS cells were seeded at a density of 10^4 cells/well in 24-well tissue culture plates. Cells were counted on days 1, 3, and 4. Data are expressed as mean \pm SD (n = 3).



Supplementary Fig. S2

Comparison of the morphology of parental and DOX-resistant OS cells.

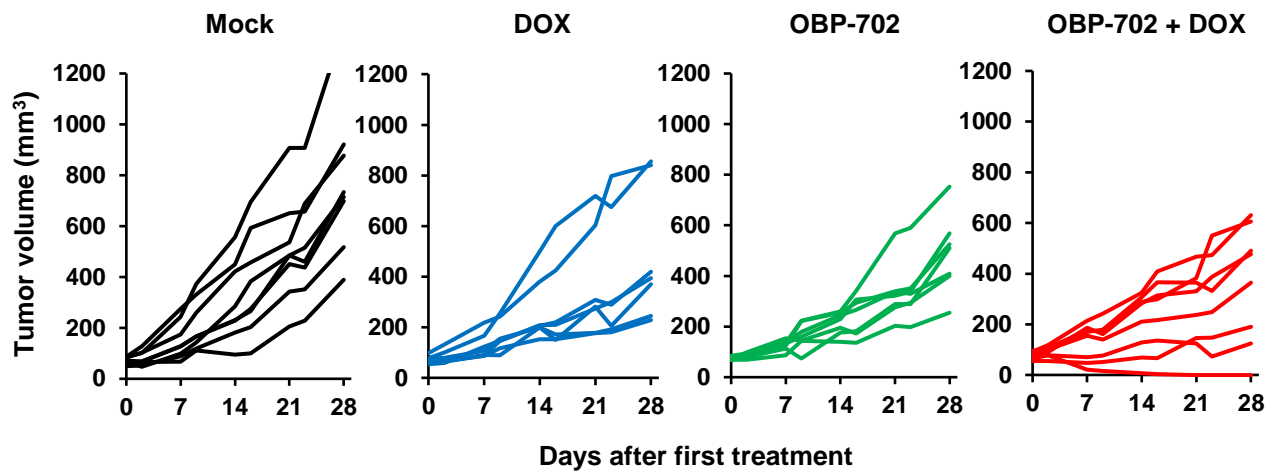
Parental and DOX-resistant U2OS and MNNG/HOS cells were seeded at a density of 10^4 cells/well in 24-well tissue culture plates. Photographs of parental and DOX-resistant OS cells were obtained under a light microscope. Scale bars, 100 μm .



Supplementary Fig. S3

Comparison of the CAR expression on the surface of parental and DOX-resistant OS cells.

Expression levels of CAR on parental and DOX-resistant U2OS and MNNG/HOS cells were assessed by flow cytometric analysis. The mean fluorescence intensity (MFI) for each cell line was determined by calculating the differences between the MFI in antibody-treated and isotype control IgG-treated cells. Data are expressed as mean \pm SD (n = 3 in each group; ** $P < 0.01$).



Supplementary Fig. S4

Tumor growth curve of individual mice bearing DOX-resistant MNNG/HOS tumors.