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Supplementary Information

Title:

Effects of gefitinib treatment on cellular uptake of extracellular vesicles in EGFR-mutant non-small cell lung cancer cells

Authors:

Tomoya Takenaka[a,b], Shinya Nakai[a,b], Miku Katayama[a,b], Mami Hirano[a,b], Natsumi Ueno[a,b], Kosuke Noguchi[a,b], Tomoka Takatani-Nakase[c], Ikuo Fujii[b], Susumu S. Kobayashi[d,e,*] and Ikuhiko Nakase[a, b,*]

Affiliations:

[a]NanoSquare Research Institute, Research Center for the 21st Century, Organization for Research Promotion, Osaka Prefecture University, Sakai, Osaka 599-8570, Japan

[b]Graduate School of Science, Osaka Prefecture University, Sakai, Osaka 599-8570, Japan

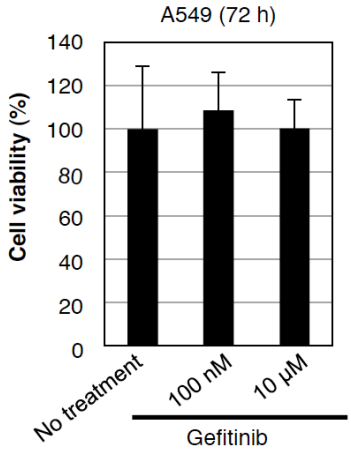
[c]Department of Pharmaceutics, School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women's University, Nishinomiya, Hyogo 663-8179, Japan

[d]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, U.S.A.

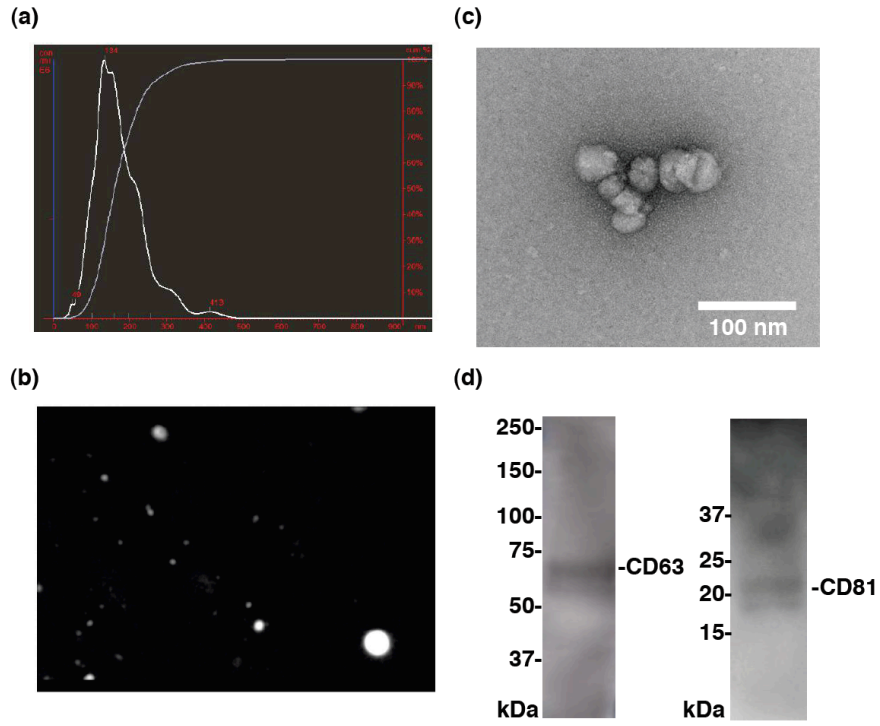
[e]Division of translational Genomics, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Kashiwa, Chiba 277-8577, Japan

*Correspondence and requests for materials should be addressed to S. S. K. (e-mail: sukobaya@east.ncc.go.jp) and to I. N. (e-mail: i-nakase@21c.osakafu-u.ac.jp)

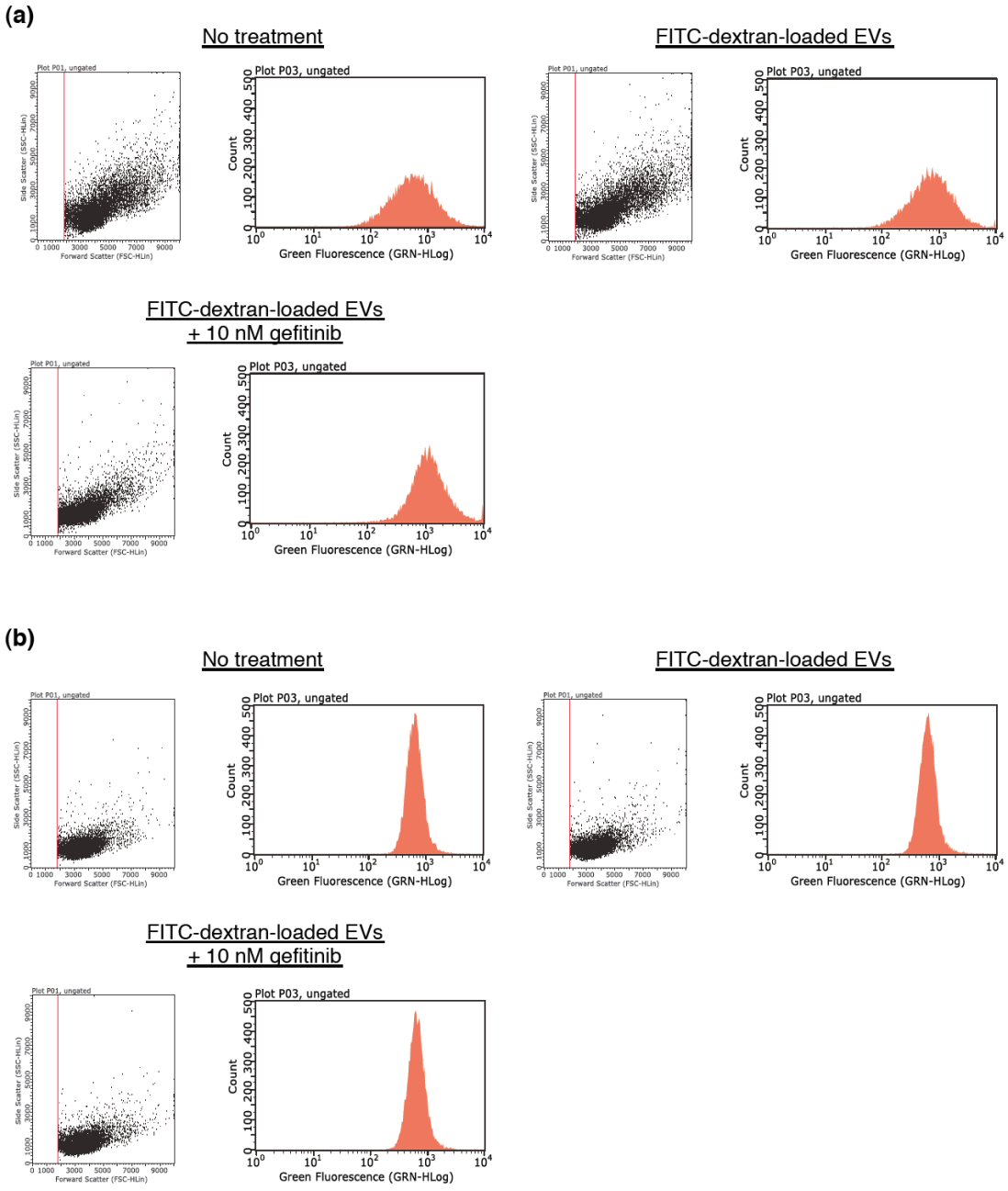
Supplementary Figures



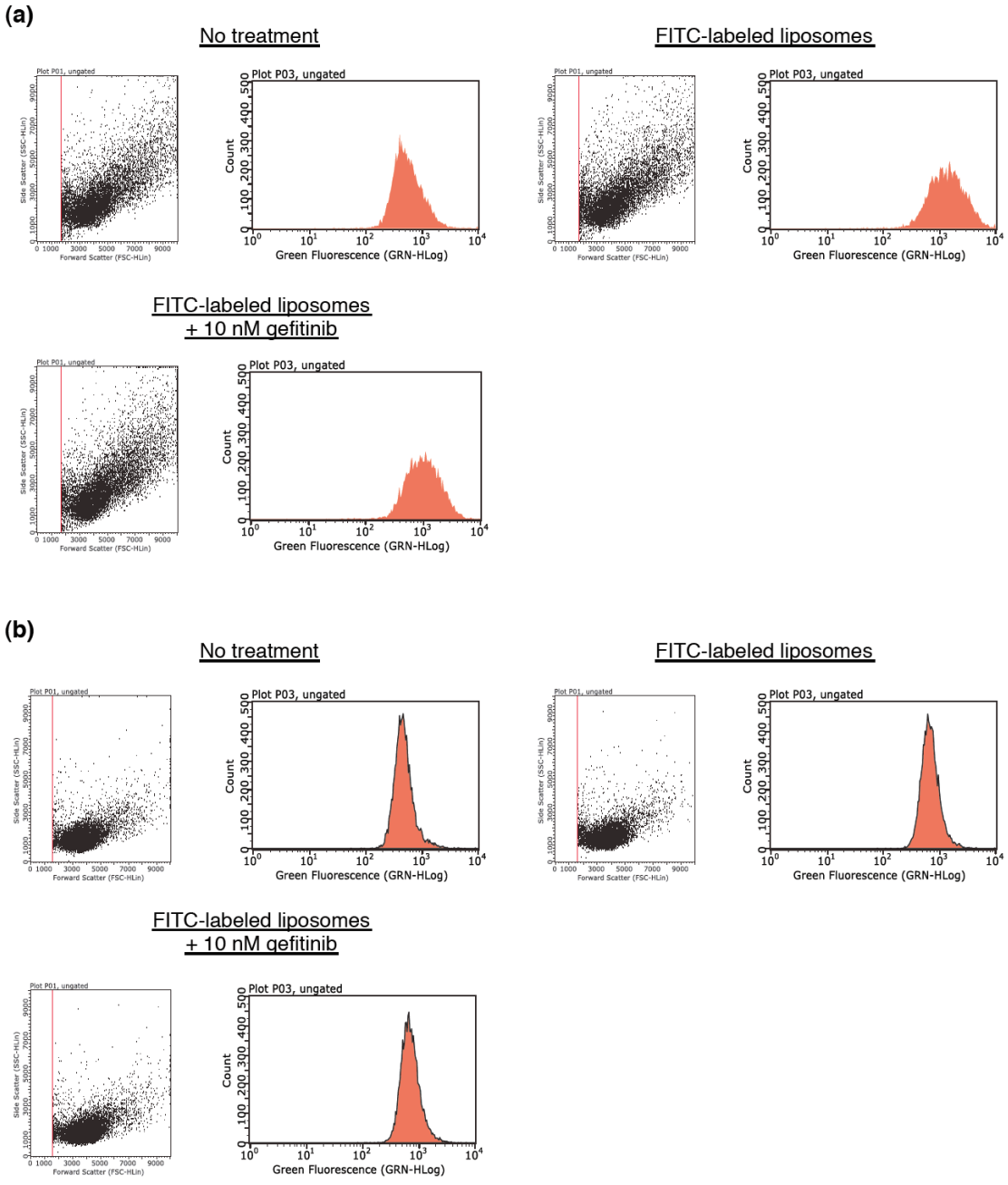
Supplementary Figure S1. Effects of gefitinib treatment on cell viability in A549 cells. Living cell counting assay of A549 cells treated with gefitinib (0 ~ 10 μM) for 72 h at 37 ˚C. The data are the averages (± SD) of four experiments.



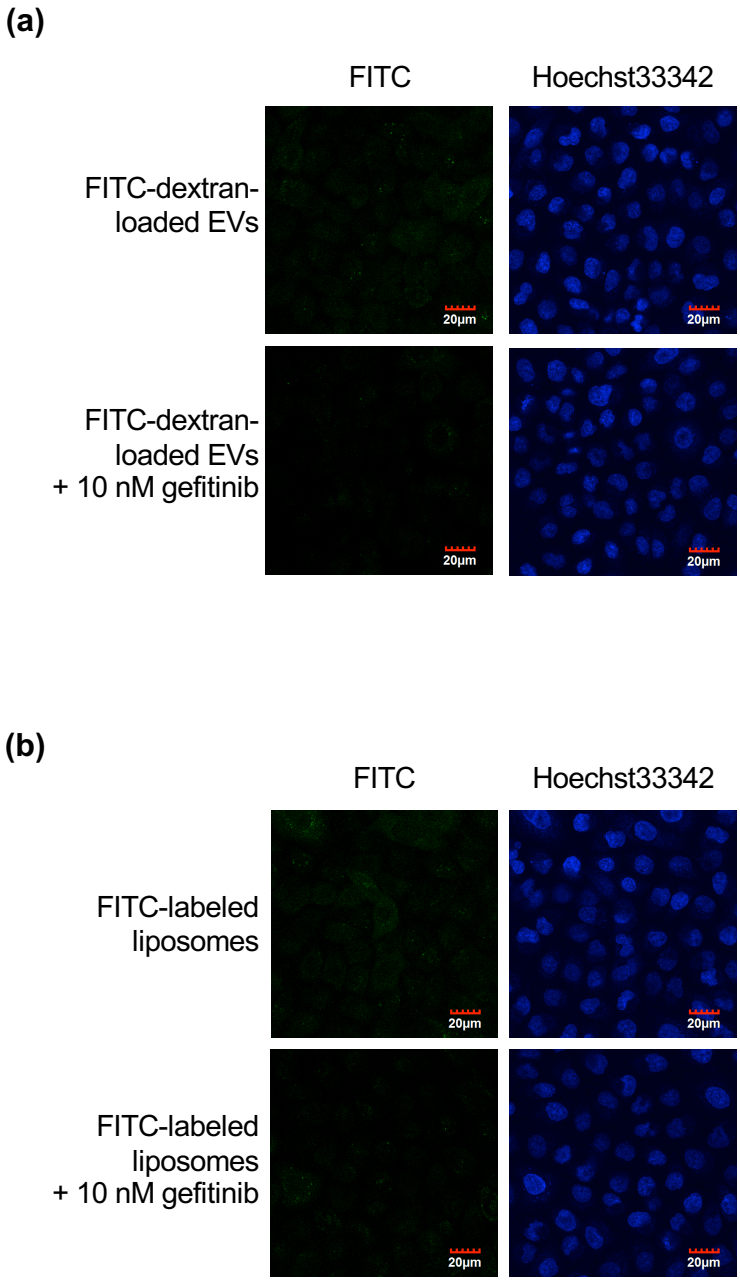
Supplementary Figure S2. Characterization of EVs. (a) Particle size distribution of HeLa EVs. (b) NanoSight video frame of HeLa EVs. (c) TEM observation of isolated EVs from HeLa cells. Scale bar: 50 nm. (d) Western blot analyses showing EVs secreted from HeLa cells. The CD63 and CD81 EV (exosome) marker proteins were detected as described in the Methods section.



Supplementary Figure S3. Effects of gefitinib treatment on cellular uptake of EVs. (a, b) Relative cellular uptake of FITC-dextran-loaded EVs in HCC827 (a) or A549 cells (b) in the presence or absence of gefitinib (10 nM) for 24 h at 37 ˚C using flow cytometry (each right figure) in the same experimental condition of Figure 2a. Living cells (10,000 cells/sample) were identified based on forward and side scatter analyses (each left figure).



Supplementary Figure S4. Effects of gefitinib treatment on cellular uptake of liposomes. (a, b) Relative cellular uptake of FITC-labeled liposomes in HCC827 (a) or A549 cells (b) in the presence or absence of gefitinib (10 nM) for 24 h at 37 ˚C using flow cytometry (each right figure) in the same experimental condition of Figure 2b. Living cells (10,000 cells/sample) were identified based on forward and side scatter analyses (each left figure).



Supplementary Figure S5. Confocal microscopic observation of A549 cells treated with EVs and liposomes. (a, b) Confocal microscopic observation of A549 cells treated with FITC-dextran-loaded EVs (a) and FITC-labeled liposomes (b) in the presence or absence of gefitinib (10 nM) for 24 h at 37 ˚C.

Supplementary Table S1. IC_{50} (μM) of DOX-loaded EVs and liposomes.

Cell line	HCC827		A549	
Gefitinib	0 nM	10 nM	0 nM	10 nM
DOX-loaded EVs	0.42	0.08	0.32	0.24
DOX-loaded liposome	0.28	0.95	0.59	0.49