Supporting Information

Membrane-Targeted Nanotherapy with Hybrid Liposomes for Tumor Cells Leading to Apoptosis

Yuji Komizu, Sayuri Nakata, Koichi Goto, Yoko Matsumoto, and Ryuichi Ueoka*

Division of Applied Life Science, Graduate School of Engineering, Sojo University,

4-22-1 Ikeda, Kumamoto 860-0082, Japan

Experimental Procedures

Preparation of hybrid liposomes. Hybrid liposomes (HL-n, n = 21, 23, 25) were prepared by the following methods.[1] L-α-dimyristoylphosphatidylcholine (DMPC) (NOF, Japan) and polyoxyethylene(*n*) dodecyl ether ($C_{12}(EO)_n$) (n = 21 and 25; Nikko Chemicals, Japan, n = 23; Sigma Chemical, U. S. A.) were mixed in 5% glucose solution and sonicated with a sonicator (VS-N300, VELVO, Japan) at 45 °C with 300 W, followed by filtration with 0.20 μm filter.

Dynamic light scattering measurement. Apparent mean hydrodynamic diameters (d_{hy}) of HL-n were measured by a dynamic light scattering method using an electrophoretic light scattering spectrophotometer (ELS-8000, Otsuka Electronics, Japan) with He-Ne laser (633 nm) as light source.[2]

WST-1 assay. Human hepatocellular carcinoma HuH-7[3] and Hep-G2, cervical cancer HeLa and lung carcinoma A549 cell lines were purchased from Riken Cell Bank (Japan). Human colon adenocarcinoma WiDr and gastric adenocarcinoma MKN45 cell lines were from Health Science Research Resources Bank (Japan). Human colon carcinoma HCT116 cell lines were from American Type Culture Collection (U. S. A.). The cells were seeded in 96 well plates (2.0×10^3 cells/well) in a

humidified atmosphere of 5% CO_2 at 37 ˚C. After 24 h, HL-n were added into each well and the plates were incubated for 48 h. The viable cell number was measured by WST-1 (2-methoxy-4-nitrophenyl-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt) assay with a Cell Counting Kit (Dojindo Laboratories, Japan) according to the manufacturer's instruction.[1] The fifty percent inhibitory concentration (IC_{50}) of HL-n was determined from the concentration-dependence of the cell viability.

TUNEL method. Apoptotic cells were detected by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling) method using an In Situ Cell Death Detection Kit (Roche Diagnostics, Switzerland).[4] After the pre-incubation of cancer cells (2.0×10^4 cells/ml) for 24 h, HL-23 was added into the culture solutions at the IC_{50} and the cancer cells were cultivated for 48 h. Subsequently, the cancer cells were stained with TUNEL and TO-PRO-3, and then the DNA fragmentation was observed with a confocal laser microscope (TCS-SP, Leica, Germany).

TIRF microscopy. Accumulation of HL-n into the plasma membranes of cancer cells was observed by total internal reflection fluorescence (TIRF) microscopy.[5,6] After the pre-incubation of cancer cells (1.0×10^5 cells/ml) for 24 h, HL-23 (1.0×10^{-4} M DMPC) containing 1-palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl-*sn*-glycero-3-phosphocholine (NBDPC) (Avanti Polar Lipid, U. S. A.) (4.6×10^{-6} M) was added into the culture solutions, and then the cancer cells were observed with a total internal reflection fluorescence microscope system (TIRFM, Olympus, Japan) equipped with an air-cooled CCD camera (EM-CCD C9100-13, Hamamatsu Photonics, Japan).

Fluorescence depolarization method. Membrane fluidity of cancer cells was evaluated on the basis of a fluorescence depolarization method with a fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) (Nacalai Tesque, Japan).[7] After the pre-incubation of cancer cells for 24 h, the cells were treated with 0.05% Trypsin/EDTA and suspended in a phosphate buffered-saline (PBS (-)), and then DPH (1.0×10^{-7}

M) was added into the cell suspension (2.0×10^4 cells/ml). The cell suspension was allowed to stand for 15 min at 37 ˚C, the fluorescence polarization (*P*) of DPH was measured using a fluorescence spectrophotometer (F-2000, Hitachi, Japan).

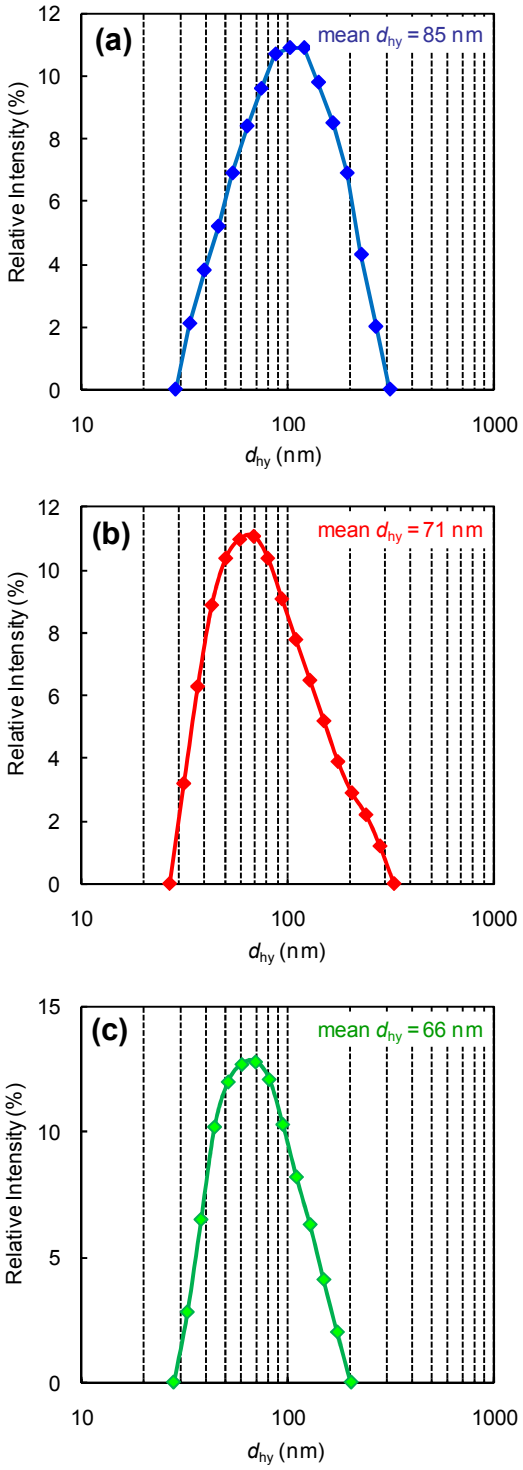

Figure S1. Distribution of hydrodynamic diameter (d_{hy}) for (a) HL-21, (b) HL-23, (c) HL -25 composed of 90 mol% DMPC and 10 mol% $C_{12}(EO)_n$ in 5% glucose solution at 25 ºC. [DMPC] = 10 mM, $[C_{12}(EO)_n]$ = 1.1 mM.

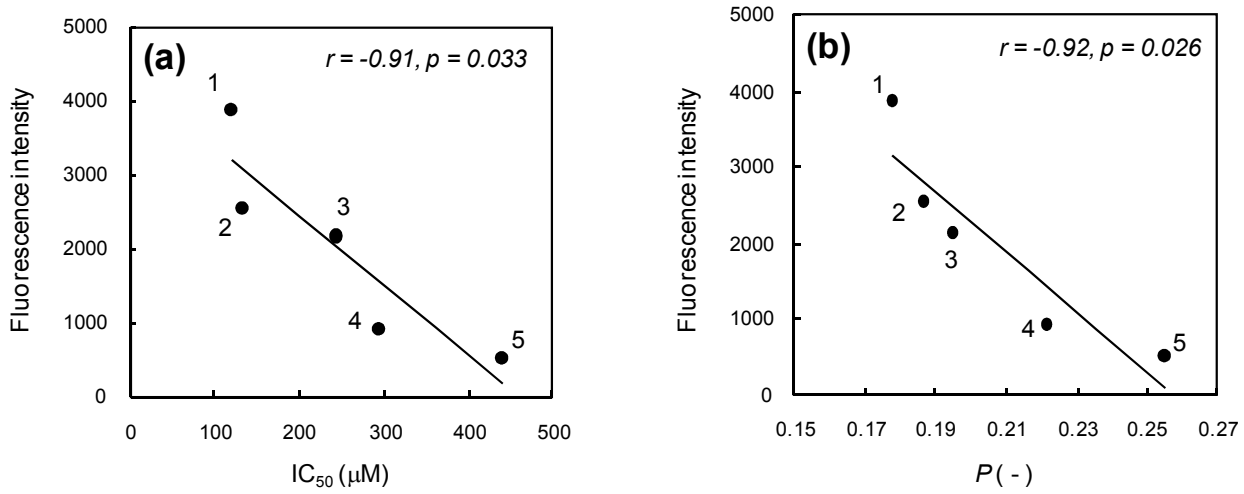

Figure S2. Correlations between (a) accumulation of HL-23 into the plasma membranes and inhibitory effects of HL-23 on the growth of tumor cells and (b) accumulation of HL-23 into the plasma membranes and the membrane fluidity of tumor cells. 1: HuH-7, 2: Hep-G2, 3: HCT116, 4: A549, 5: HeLa cells.

Table S1. Fluorescence polarization (*P*) of DPH in the plasma membranes of various cancer cells[a]

cancer cell	*P* (-)
HuH-7 (Liver)	0.178 ± 0.002
Hep-G2 (Liver)	0.187 ± 0.003
MKN- 45 (Stomach)	0.196 ± 0.002
HCT116 (Colon)	0.195 ± 0.004
WiDr (Colon)	0.201 ± 0.003
A549 (Lung)	0.222 ± 0.004
HeLa (Cervix)	0.255 ± 0.001

[a] Data represented are the mean ± S. E..

References

(1) Matsumoto, Y.; Iwamoto, Y.; Matsushita, T.; Ueoka, R. Novel Mechanism of Hybrid Liposomes-Induced Apoptosis in Human Tumor Cells. *Int. J. Cancer* **2005**, *115*, 377-382.

(2) Iwamoto, Y.; Matsumoto, Y.; Ueoka, R. Induction of Apoptosis of Human Lung Carcinoma Cells by Hybrid Liposomes Containing Polyoxyethylenedodecyl Ether. *Int. J. Pharm.* **2005**, *292*, 231-239.

(3) Nakabayashi, H.; Taketa, K.; Miyano, K.; Yamane, T.; Sato, J. Growth of Human Hepatoma Cells Lines with Differentiated Functions in Chemically Defined Medium. *Cancer Res.* **1982**, *42*, 3858-3863.

(4) Shimoda, S.; Ichihara, H.; Matsumoto, Y.; Ueoka, R. Chemotherapy with Hybrid Liposomes for Human Breast Tumors along with Apoptosis *in Vivo*. *Int. J. Pharm.* **2009**, *372*, 162-178.

(5) Komizu, Y.; Matsumoto, Y.; Ueoka, R. Membrane Targeted Chemotherapy with Hybrid Liposomes for Colon Tumor Cells Leading to Apoptosis. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6131-6134.

(6) Axelrod, D. Total Internal Reflection Fluorescence Microscopy. *Methods Cell. Biol.* **1989**, *30*, 245-270.

(7) Ngami, H.; Matsumoto, Y.; Ueoka, R. Chemotherapy with Hybrid Liposomes for Lymphoma without Drugs *in Vivo*. *Int. J. Pharm.* **2006**, *315*, 167-172.