Supporting Information

Fluorescent Nanoparticles for Targeted Tumor Imaging and DNA Tracking Gene Delivery in vitro/in vivo

Wan Sun [a, b], Fang Tang [b], Jing-Xue Cui [b], Zhong-Lin Lu[b*]

[a] Shandong Provincial Engineering Laboratory of Novel Pharmaceutical Excipients, Sustained and Controlled Release Preparations, College of Medicine and Nursing, Dezhou University, Dezhou 253023, China

[b] Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, China

* E-mail: luzl@bnu.edu.cn

1. Materials and apparatus

^{1}H and ^{13}C NMR spectra at 25 °C using deuterated dimethylsulfoxide (DMSO-d_6) and chloroform ($CDCl_3$) as solvents were recorded from a Bruker Avance III 400 or 500 MHz spectrometer (Bruker, Germany) and calibrated using tetramethylsilane (TMS) as an internal reference. High resolution mass spectra (HRMS) were acquired by Waters LCT Premier XE spectrometer (Waters, USA). Fluorescence studies were carried out with Varian Cary Eclipse spectrometer (Agilent, USA). UV-Vis spectra carried out with Varian Cary 300 UV-Vis spectrophotometer (Agilent, USA). Fluorescent images were acquired on confocal laser scanning microscopy (CLSM) Nikon A1R systems (Japan). Flow cytometry were acquired on CytoFLEX (Beckman, USA). EGFP and pGL-3 DNA were synthesized by Ruibiotech Co., Ltd. (Beijing, China). Tumor cells and mice were obtained from the Institute of Biochemistry and Cell Biology, SIBS, CAS (China), All animal experiment protocols were approved by animal care committee of Beijing Normal University.

2. Agarose Gel retardation assay

The condensing abilities of **DPL** toward plasmid DNA (pUC-18) were evaluated using agarose gel electrophoretic analysis. **DPL** and pUC-18 at various concentration (2.5, 5, 10, 20, 40 μM) were first prepared by gently vortexing the mixtures of pUC-18 solution (10 μg/mL) and allowing to incubate for 30 min at 37 ^{o}C. The complexes were electrophoresed on a 0.7 % (w/v) agarose gel containing Golden view II in Tris-acetate (TAE) running buffer at 150 V for 30 min. Then DNA was visualized under an ultraviolet lamp using a Vilber Lourmat imaging system.

3. In vitro cytotoxicity assay

The relative toxicity of the **DPL** was characterized by MTT assay. A549、HepG2、Hela、 MCF-7 were seeded in to 96-well at a density of 6×10^3 cells, and incubated under 37 ^{o}C and 5% CO_2 for 12 h. Then, **DPL** at different concentrations were added into the cell dishes and then incubated for another 24 h. The medium was removed and the cytotoxicities were evaluated using the MTT assay. Finally, the absorbance was recorded and the cell viability was calculated.

4. Transfection efficacies of DPL on different cell lines

Four kinds of cells were seeded in a 24-well culture plate at a density of 4×10^4 cells per well for 24 h. Generally, 10 μg/mL EGFP or luciferase plasmids were mixed with the different concentrations of **DPL** (10, 20, 30, 40, 50, 60 μM) for 30 min. Lipo 2000, a commercial transfection reagent, was used as a contrast vector (10μM). The cells were incubated with the new complex in DMEM. After 5 h, 500 μL fresh DMEM (10% FBS) was added to each well and the cells were further incubated for 24 h. EGFP expressions were examined by CLSM and flow cytometry. Luciferase expressions were measured according to the Luciferase detection kit. The luciferase activities were normalized with respect to protein concentrations (relative light units per mg of protein, RLU/mg protein). The protein concentration in each well was measured by a BCA kit (Beyotime).

5. In vivo imaging

When the tumor grew to 70-80 mm^3, three nude mice were treated by intra-tumoral injection of 50 μM **DPL**/DNA, the fluorescent imaging studies and biodistribution in vivo were determined using tumor-bearing nude mice by the in vivo imaging system.

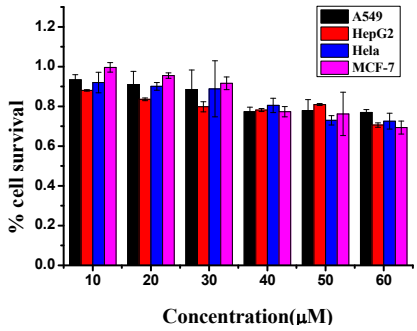

Figure S1 In vitro cytotoxicity of **DPL** against A549、HepG2、Hela、MCF-7 cells over 24 h

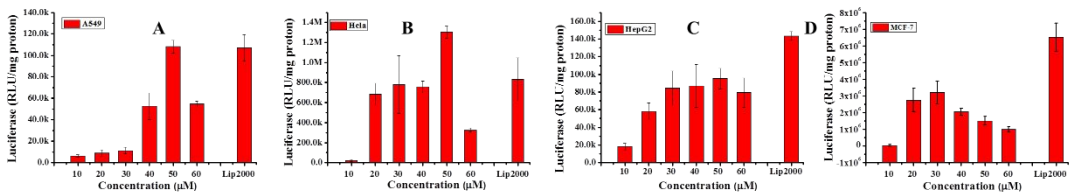

Figure S2 Transfection efficiencies of pGL-3 DNA by **DPL** at varied concentrations in four cells

by luciferase assays [pGL-3 DNA]=10 μg/Ml

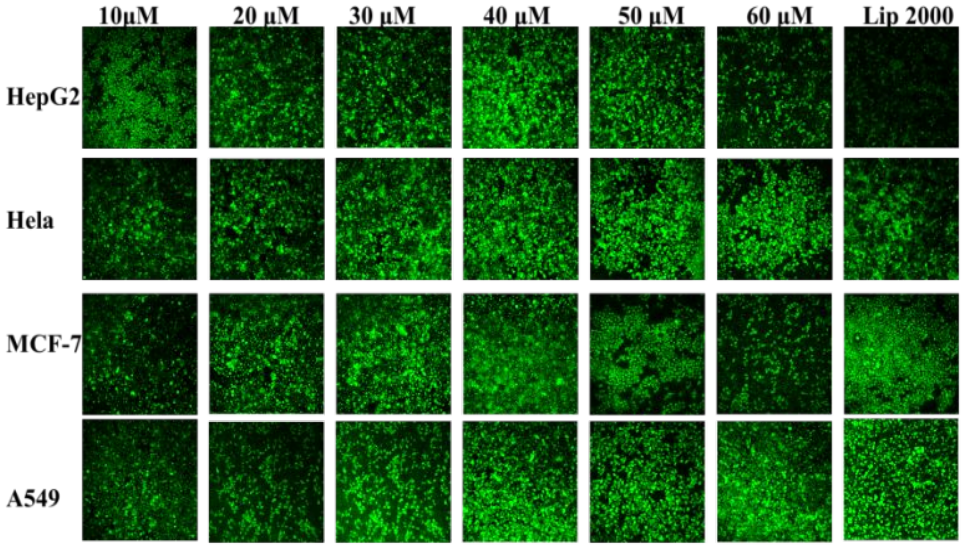

Figure S3 Transfection efficiencies of GFP DNA by **DPL** at varied concentrations in four cells by luciferase assays [GFP DNA]=10 μg/mL