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Supporting information

Self-assembled fluorodendrimers in the co-delivery of fluorinated drugs and therapeutic genes

Hui Wang[a,b], Jingjing Hu[a], Xiaopan Cai[b], Jianru Xiao*[b], Yiyun Cheng*[a]

[a]*Shanghai Key Laboratory of Regulatory Biology, School of Life Sciences, East China Normal University, Shanghai, 200241, P. R. China*

[b]*Changzheng Hospital, Department of Orthopedic Oncology, Shanghai, P. R. China*

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Experimental section

Materials

Ethylenediamine-cored and amine-terminated G2 polyamidoamine dendrimer (molecular weight: 3254 Da) was purchased from Dendritech, Inc. (Midland, MI). Heptafluorobutyric anhydride, butyric anhydride, sorafenib and 5-fluorouracil (5-Fu) were purchased from Sigma-Aldrich (St Louis, MO). Lipofectamine 2000 (Lipo 2000) was purchased from Life Technologies (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sangon (Shanghai, China). SB202190 was a gift from Selleckchem (Houston, TX). The molecular weight and purity of G2 polyamidoamine dendrimer was determined by mass spectroscopy and ^{13}C NMR. The chemicals were used as received without further purification.

Synthesis and characterization of fluorodendrimers

The fluorodendrimers were synthesized by reacting G2 polyamidoamine dendrimer with heptafluorobutyric anhydride. Briefly, heptafluorobutyric anhydride and G2 dendrimer were dissolved in methanol at different molar ratios (8:1, 10:1 and 12:1, respectively). Triethylamine (1.25 molar equivalents of heptafluorobutyric anhydride) was immediately added to neutralize the yielding acids. The mixtures were stirred at room temperature for 48 h. After that, excess diethyl ether was added into the mixture and the solid products were collected. The products were further purified by re-precipitation in diethyl ether twice and dried under vacuum at 40 °C for 12 h to obtain fluorodendrimers as light yellow gels. The residual primary amine groups on fluorodendrimers were measured by a ninhydrin assay as described elsewhere.

Butyric acid modified G2 polyamidoamine dendrimer was synthesized as described above. The molar ratio of butyric anhydride to G2 dendrimer is 12:1. The number of butyric acids conjugated on each G2 dendrimer was determined by both ^{1}H NMR (Varian, 699.804 MHz) and ninhydrin assay..

Preparation of fluorinated drug-loaded fluorodendrimers

The fluorinated drugs and fluorodendrimers were dissolved in dimethyl sulphoxide at a concentration of 80 mM, and then added dropwise into distilled water. The final concentration of the drugs is 0.8 mM. The mixture was maintained at room temperature for 1 h before further experiments.

The sorafenib loading capacity of the fluorodendrimers

Fluorodendrimers (0.1 μmol) and sorafenib were dissolved in 10 μL dimethyl sulphoxide. Drug-loaded fluorodendrimers were formed by adding the solutions into 1 mL water followed by gentle vortexing. The initial amount of sorafenib in the mixture solutions was 0.1 μmol, and then gradually increased until the yielding mixture solution became turbid. The number of sorafenib loaded per fluorodendrimer (NSFD) and the drug loading efficacy (DLE) were calculated according to the components in the saturated solution.

NSFD= (mole number of drug/mole number of polymer in the complex solution) *100%

DLE (%)=[weight of drug in the complex solution/weight of polymer and drug in the complex solution]*100%

Characterizations of the self-assembled nanostructures

The synthesized fluorodendrimer G2-$F7_{12}$, G2-$F7_{12}$/sorafenib and G2-$F7_{12}$/5-Fu complexes were dissolved in distilled water and the assembled nanostructures on carbon grids were examined by TEM (JEOL JEM-2100). Sizes of the assembled nanostructures were further characterized using DLS at 25 °C (Zetasizer NanoZS90, Malvern).

In vitro release of fluorinated drugs from the fluorodendrimer

In vitro drug release was investigated by an equilibrium dialysis method. Sorafenib-loaded G2-$F7_{12}$ complex solution (0.178 mM sorafenib, the molar ratio of G2-$F7_{12}$ and sorafenib is 1:1) was transferred into a dialysis bag with a molecular weight cut off (MWCO) of 3500 Da. Then the dialysis bag was immersed into a container filled with 50 mL PBS buffer (pH=7.4). The outer phase of the dialysis bag was stirred at 300 rpm during the release studies. 10 μL of the samples were collected at scheduled time intervals from the outer phase and sorafenib concentration in the collected sample was determined by high-performance liquid chromatography (HPLC, Agilent1200) at the wavelength of 270 nm. The HPLC instrument was equipped with a C18 column (4.6 mm diameter, 150 mm length, 5 μm particle size, ZORBAX Eclipse XDB, Agilent, USA). The mobile phase was acetonitrile and PBS buffer (pH=7.4) (55%:45%, v/v) at a flow rate of 1.0 mL/min. The standard curve for sorafenib was conducted at a sorafenib concentration range of 0-10 μg/mL (A = 20.547C−0.213, C is the sorafenib concentration, μg/mL, A is the peak area, R2= 0.9997). For 5-Fu, the mobile phase was methanol and deionized water (10%:90%, v/v) and the standard curve was A = 20.606C+0.822, C is the 5-Fu concentration, μg/mL, A is the peak area, R2= 0.998). The release of fluorinated drugs from unmodified G2 polyamidoamine

dendrimer and G2-BA_{12} were measured as controls.

Cell culture, in vitro gene transfection and cytotoxicity assay

MDA-MB-231 cells (a human breast adenocarcinoma cell line, ATCC) were cultured in MEM at 37 °C and 5% CO_2. The cell culture media were supplemented with 10% fetal bovine serum (FBS), 100 μg/mL streptomycin, and 100 units/mL penicillin.

The cells were cultured in 24-well plates for 24 h before in vitro gene transfection (70-80% confluent), and then incubated with G2-$F7_{12}$/EGFP plasmid or G2-$F7_{12}$/sorafenib/EGFP plasmid polyplexes (0.5 μg EGFP plasmid in each well, the N/P ratio is 4, the sorafenib concentration is 4 μM) for 6 h. After that, 500 μL fresh MEM containing 10% FBS were added and the transfection were continued for another 42 h. The expression of EGFP in MDA-MB-231 cells was observed by a fluorescent microscopy (Olympus, Japan) and quantitatively measured by flow cytometry (BD FACSCalibur, San Jose). Three repeats were conducted for each transfection.

The cytotoxicity of G2-$F7_{12}$/TRAIL plasmid complexes were measured by a well-established MTT assay. Generally, MDA-MB-231 cells were seeded in 96-well plates with a density of 10000 cells per well one day before the experiment. Cells were washed with PBS once, and then were incubated with G2-$F7_{12}$/TRAIL plasmid complexes at different N/P ratios (the G2-$F7_{12}$ concentration is kept constant at 4 μM) at 37 °C for 48 h. Cells without treatment were tested as a control. A standard MTT assay was used to determine the cytotoxicity. Five repeats were conducted for each material. The results were analysed by student's t-test.

For co-delivery experiments, the toxicity of G2-$F7_{12}$/sorafenib/TRAIL plasmid complexes on MDA-MB-231 cells was determined by the same MTT assay. The concentrations for sorafenib and G2-$F7_{12}$ are 4 μM. The N/P ratio of G2-$F7_{12}$/TRAIL plasmid is 4. Free sorafenib, G2-$F7_{12}$/sorafenib, G2-$F7_{12}$/TRAIL plasmid, and Lipo 2000/sorafenib/TRAIL plasmid complexes were tested as controls. The optimal dose of Lipo 2000 is screened to be 1.5 μL per well. To increase the sensitivity of MDA-MB-231 cells to sorafenib, 20 μM SB202190 was added in each well.

For the G2-$F7_{12}$/5-Fu/TRAIL plasmid complexes in the MTT assay, the concentrations for 5-Fu and G2-$F7_{12}$ are 64 μM and 4 μM, respectively. The N/P ratio of G2-$F7_{12}$/TRAIL plasmid is 4.

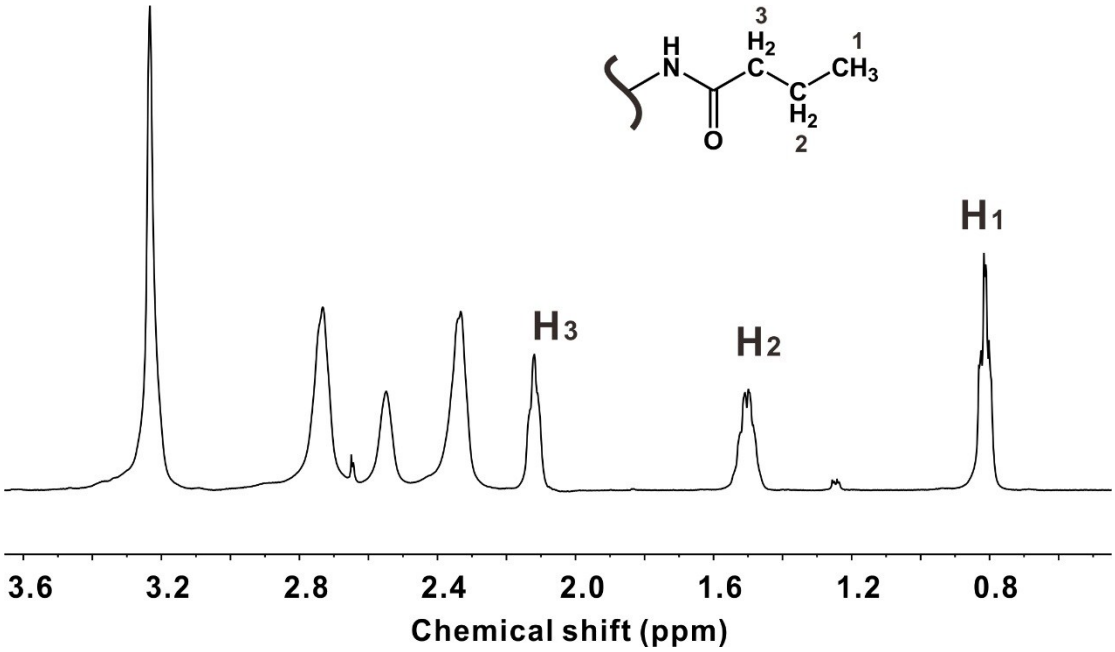

Figure S1. (a) ^{1}H NMR spectrum of butyric acid-modified G2 polyamidoamine dendrimer. According to ^{1}H NMR, an average number of 12.3 butyric acid groups were conjugated on each G2 dendrimer. The product was defined as G2-BA_{12}.

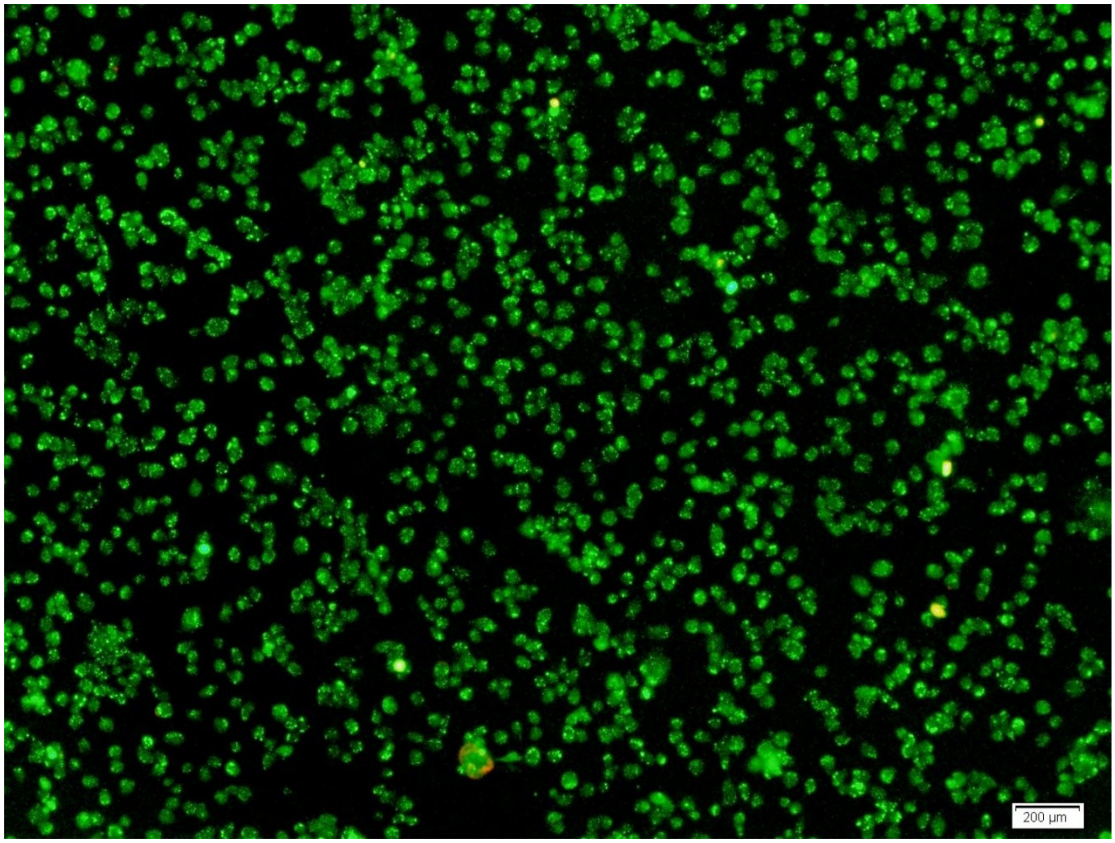

Figure S2. Cytotoxicity of 4 µM G2-$F7_{12}$ on MDA-MB-231 cells by an acridine orange/ethidium bromide double-staining assay. The cells were incubated with the fluorodendrimer for 48 h before the assay. The cells were washed twice with cold PBS after incubation with fluorodendrimers, and then were stained with acridine orange (5 µg/mL) and ethidium bromide (5 µg/mL) for 3 min. After that, the cells were again washed twice with cold PBS and observed by a fluorescent microscope.