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Electronic Supplementary Information

Synthesis of glycoconjugated poly(amindoamine) dendrimers for targeting human liver cancer cells

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Experimental Section

Materials. Ethylenediamine core amine-terminated poly(amidoamine) (PAMAM) dendrimers of generation 5 (G5.NH_2) with a polydispersity index less than 1.08 were purchased from Dendritech (Midland, MI). Lactobionic acid (La), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich (St Louis, MO) and used as received. Acetic anhydride, methanol, triethylamine, dimethyl sulfoxide (DMSO), and other solvents were of analytical grade and used without further purification. The water used in all the experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with resistivity higher than 18 MΩ.cm. Regenerated cellulose dialysis membranes (molecular weight cut-off, MWCO = 10000) were acquired from Fisher.

***Synthesis of G5-FI-Ac and G5-FI-Ac-La conjugates*.** G5.NH_2 dendrimer was conjugated with FI according to previous published methods.[1] Briefly, G5.NH_2 (30 mg, 0.00115 mmol) was dissolved in anhydrous DMSO (20 mL). Then a solution of FI (2.25 mg, 0.00575 mmol) in DMSO (10 mL) was added dropwise at room temperature under vigorous stirring for 24 h. The mixture was dialyzed against PBS buffer (3 times 4 L) and water (3 times 4 L) for 3 days through a 10000 MWCO membrane, followed by lyophilization to give G5.NH_2-FI conjugates as an orange solid.

The G5.NH_2-FI conjugates were divided into two parts. The first part was acetylated to convert all remaining amino groups of G5 dendrimer to acetamide groups (G5-FI-Ac). Briefly, G5.NH_2-FI was dissolved in DMSO and mixed well with triethylamine. Then, acetic anhydride solution (120 molar equivalents, DMSO) was added dropwise to the dendrimer/triethylamine mixture solution under vigorous stirring for 24 h. Followed by dialysis and lyophilization, G5-FI-Ac was obtained.

The second part of G5.NH_2-FI product was partially acetylated to convert 90 amino groups of dendrimer to acetamide groups (G5-FI-Ac-NH_2) according to a procedure described above, by controlling the molar ratio of acetic anhydride to dendrimer at 90:1. After extensive dialysis and lyophilization, G5-FI-Ac-NH_2 was formed. Then, the G5-FI_5-Ac-NH_2 product was coupled with La molecules via amide bonds between carboxyl groups of La molecules and the amine groups on the surface of dendrimers. Briefly, a 10-molar equivalent of La was activated by NHS/EDC dissolved in 5 mL of PBS buffer (pH 6.0) for 4 h. EDC was 4-fold molar excess over

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La and NHS/EDC molar ration was set at 1:1. The activated La solution was added to the G5-FI-Ac-NH_2 solution and allowed to react with stirring for 3 d at room temperature. Then, the mixture solution was dialyzed against PBS buffer and water to remove the excess reactants and byproducts, and lyophilized to obtain the product G5-FI-Ac-La.

Characterization. ^{1}H NMR spectra of G5-FI-Ac and G5-FI-Ac-La were recorded on a Bruker AV400 nuclear magnetic resonance spectrometer. Samples were dissolved in D_2O before NMR measurements. MALDI-TOF spectra were acquired using a Micromass TofSpec-2E spectrometer. Linear mode was selected as the operation mode. 10 mg mL^{-1} beta-indoleacrylic acid in acetonitrile/H_2O (v/v = 70:30) was used as the matrix. One mg dendrimer samples were dissolved in 1 mL methanol and then diluted 5 times by methanol to get the final concentration of 0.2 mg mL^{-1}. An equal volume of 0.2 mg mL^{-1} dendrimer solution and matrix solution were well mixed. Then, one μL solution of the mixture was injected on the spots of the target plate. Five picomole proteins of cytochrome-C (12 359 g mol^{-1}), myoglobin (16 951 g mol^{-1}), and trypsinogen (23 976 g mol^{-1}) were used as external standards.

Cytotoxicity analysis of the conjugates. Cytotoxicity of the prepared conjugates was evaluated in vitro using standard MTT colorimetric assay. Human liver carcinoma cell line HepG2 cells (Shanghai Institute of Cell Biology, China) were seeded in 96-well plates at a density of 1.5×10^4 cells/well. After overnight incubation, the growth medium was replaced with G5-FI-Ac-La and G5-FI-Ac conjugate solutions at concentrations ranging from 0 to 2000 nM. The cells were incubated for 24 h at 37 ℃

and washed twice by 1 mL PBS buffer. Subsequently, 20 μL of a 10 mg/mL MTT (Sigma, St. Louis, MO) solution was added to each well, and the plate was incubated for an additional 4 h, allowing the viable cells to reduce the yellow MTT into dark blue formazan crystals. Finally, 200 μl of DMSO were added into each well to dissolve the crystals, and the absorbance of individual well was measured by a Microplate Reader (Multiskan MK3, Thermo, USA). Cell viability was determined by the following equation: Cell viability (%) = $Abs_{\text{test cells}}/Abs_{\text{reference cells}} \times 100\%$.

Flow cytometry analysis. Approximately 1×10^6 HepG2 cells per well were seeded in 12-well plates the day before the experiments. G5-FI-Ac-La and G5-FI-Ac conjugates were added in each well respectively, with the final concentrations at 0, 50, 100, 200, 300, 500, 800, and 1000 nM. After 2 h of incubation with the conjugates, HepG2 cells were trypsinized (Gibco/BRL, Gaithersburg, MD) and suspended in PBS buffer containing 0.1% bovine serum albumin (Sigma, St. Louis, MO) and analyzed using a Becton Dickinson FACScan Flow Cytometer. The FL1-fluorescence of 10000 cells was measured, and the mean fluorescence of gated viable cells was quantified.

Confocal microscopy. Confocal microscopic analysis was performed using a Carl Zeiss LSM 710 Confocal Microscope (Thornwood, NY). Cells plated on a plastic cover-slip were imaged. FI fluorescence was excited with a 488 nm argon blue laser and emission was measured through a 505–525 barrier filter. The optical section thickness was set at 5 μm. The HepG2 cells or MCF-7 cells were incubated with PBS solution (control), G5-FI-Ac-La, and G5-FI-Ac, respectively with a final conjugate concentration of 500 nM for 2 h, followed by rinsing with PBS buffer. The nuclei

were counterstained with 1 μg/mL of Hoescht33342, using a standard procedure. Samples were scanned using a 63× water-immersion objective lens.

References

(1) Shi, X.; Wang, S. H.; Meshinchi, S.; Van Antwerp, M. E.; Bi, X. D.; Lee, I. H.; Baker, J. R., Jr. *Small* **2007**, *3*, 1245.

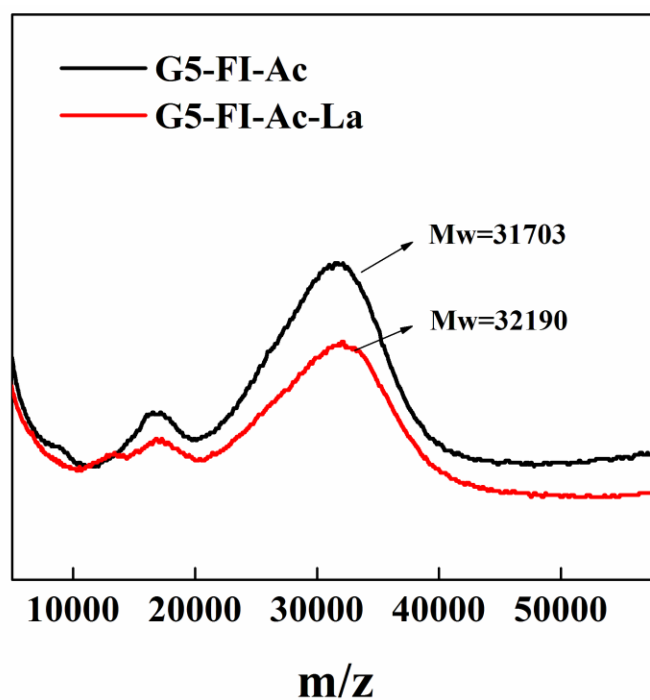

Fig.S1. MALDI-TOF mass spectra of G5-FI-Ac and G5-FI-Ac-La conjugates.