Supporting Information For

Folate-modified bexarotene-loaded bovine serumalbumin nanoparticles as a promising tumor-targeting delivery system

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Cell culture

The two cell lines including human non-small-cell lung cancer cells of A549 and human breast cancer cells lines of MCF-7 were cultivated in 75 cm^3 flasks at 37℃ in a humidified atmosphere with 5% CO_2. Cells were cultured in normal or folate-free RPMI medium, supplemented with10% fetal bovine medium (FBS). Cells were subcultured every other day. Before experiment, the cells were cultured until confluence was reached to about 75%. Since free folate can compete and downregulate the folate receptors on the cell surface, the cell toxicity and uptake experiments were conducted with folate-free mediums directly before each assay.

MTT assay

In brief, A549 and MCF-7 cells in their logarithmic growthwere cultured in 96-well plates with a density about 5000 cells/well (0.1 mL medium). Cells were adherent overnightand cultured with fresh media containing serial dilutions of treatment drugs including free bexarotene solution, BEX-BSANPs and FA-BEX-BSANPs. The concentrations of bexarotene in the present study were ranging from 0.1 μM to 150 μM (0.1 μM, 1 μM, 10 μM, 50 μM, 100 μM, 150 μM).

The viability of the cells was tested using MTT assay at scheduled time intervals.Untreated cells served asreference and were taken as 100% viability. Each sample was performed in sextuplicate. Then 20 μL of 5 mg/mL MTT dissolved in PBS was added to each well. The plates were incubated for another 4 h at 37℃ and then the medium was discarded. Thereafter, 200 μL of DMSO was added to each well to dissolve the formed purple crystals derived from MTT with vigorously stirring the plates. The absorbance of the wells was tested by the microplate reader (Bio-Rad680, USA) at 570 nm wavelength.

Meanwhile, the cytotoxicity of the blank nanoparticles in the absence of BEX was also tested with the same method with concentration of BSA ranging from 0.8 to 800

μg/mL.

Propidium iodide staining process

A549 cells and MCF-7 cells (2×10^5cells/well) were seeded into 6-well culture plates and incubated with free BEX solution, BEX-BSANPsand FA-BEX-BSANPs containing total BEX concentration of 20 μM for 24 h.After washed with PBS three times, the cells were collected and stained with PI for 15 min in dark at 37℃.

The cellular uptake and flow cytometric (FCM) analysis

The cells were cultured as described above for cellular uptake studies. A549 and MCF-7 cells were seeded on six-well culture plates (2×10^5 cells/well, folate-free medium) and allowed to grow overnight. The cells were incubated in the fresh folate-free medium at 37℃ with different treatments (with an equivalent coumarin-6 concentration of 10 μg/mL). After an incubation period of 2 hours extracellular coumarin-6 was removed by aspirating the supernatant followed by washing three times with phosphate buffered saline (PBS). The cells were imaged using inverted fluorescence microscope (OLYMPUS, Japan).

Flow cytometry was utilized to quantify the cellular uptake. The cells were harvested by trypsinization with centrifugation (1000 rpm, 3 min). Finally, the cells were suspended in 200 μL of PBS and stored on ice until analysis. The fluorescence intensity in the cells was determined using a flow cytometer (Becton Dickinson, San Jose, CA). The number of cells collected was ten thousand, and the experiments were run in triplicate.

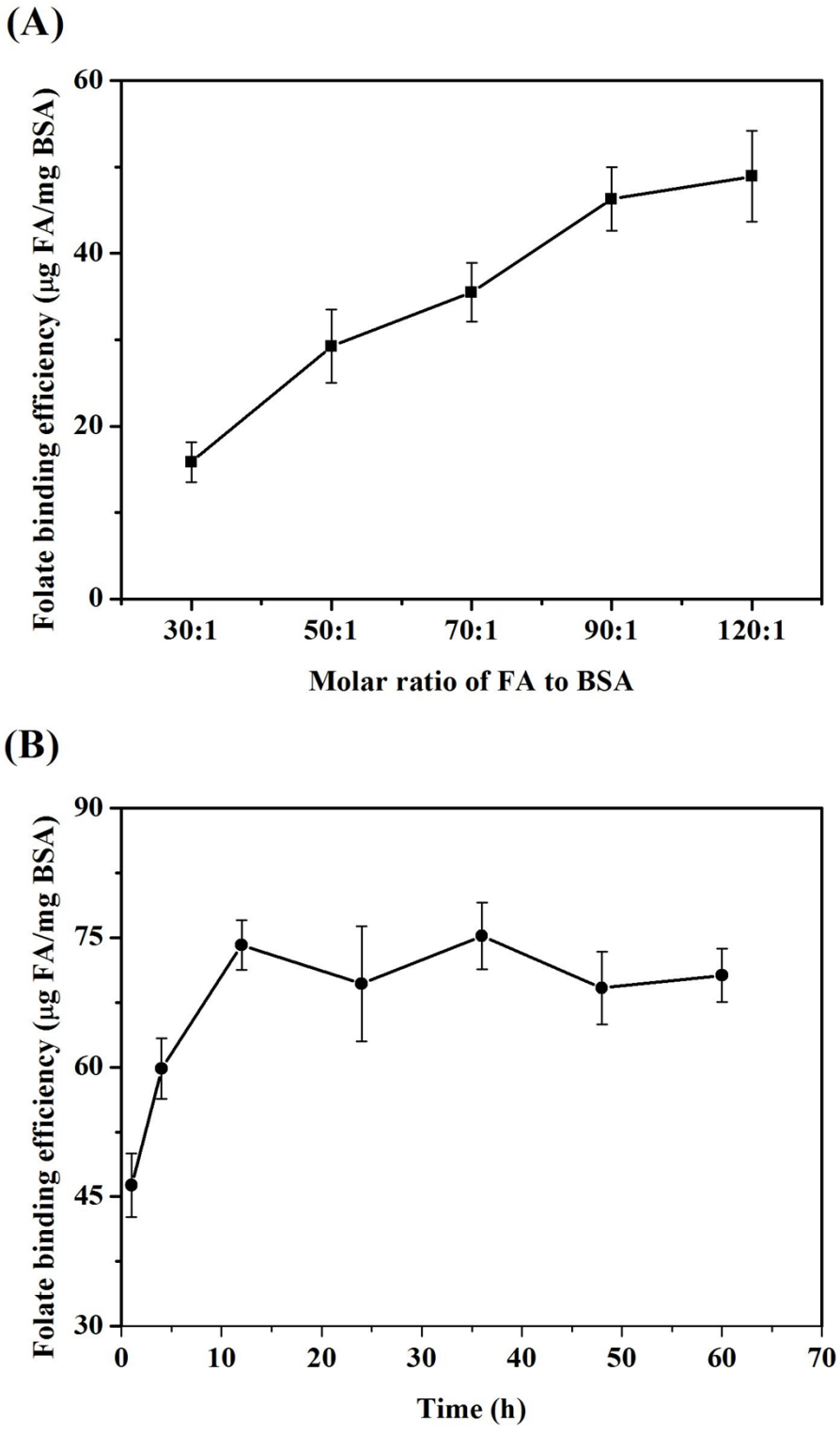

Fig. S1. Influence of the molar ratio of FA to BSA (A) and reaction time (B) on the folate content conjugating to BSA. (n = 3)

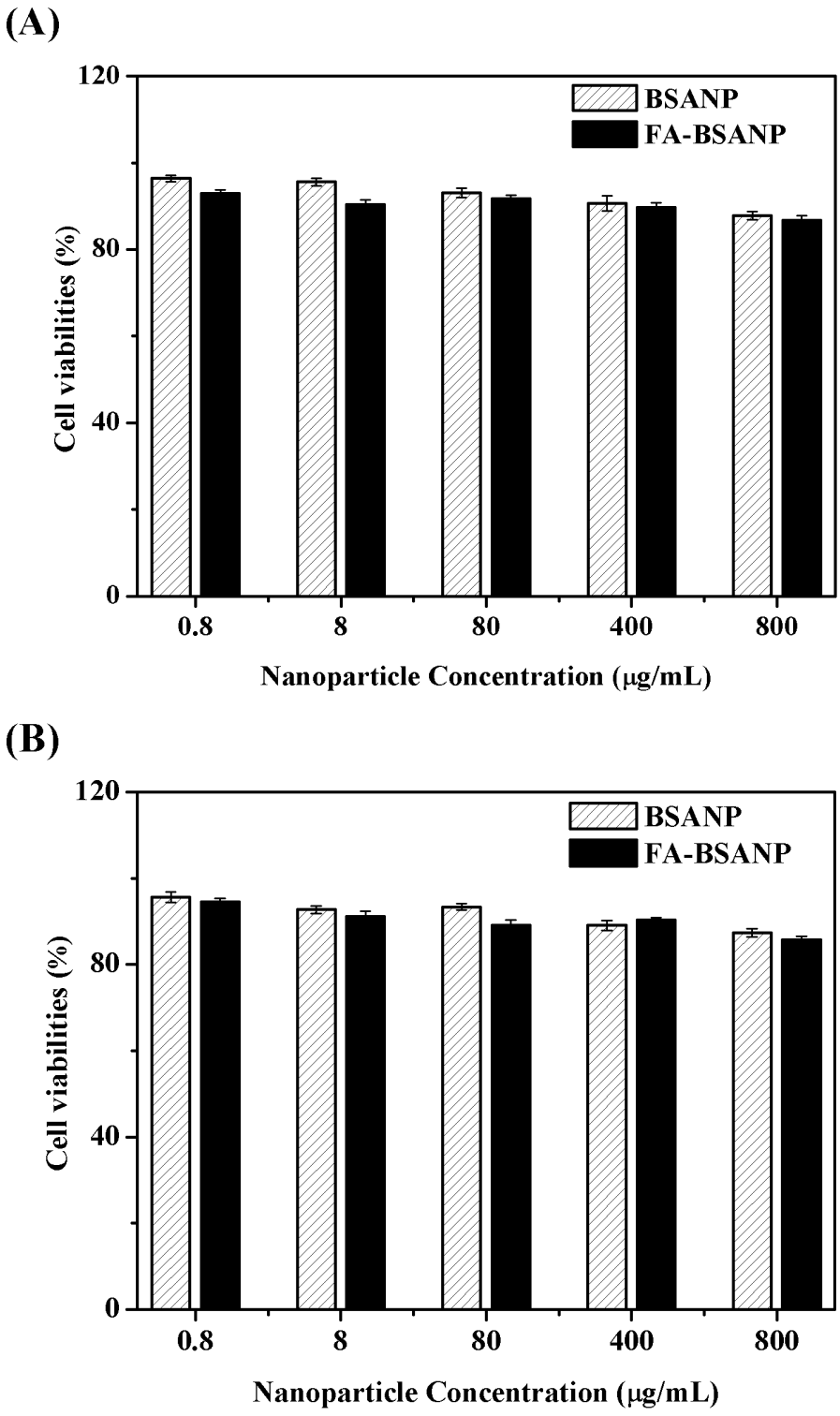

Fig. S2. Cell viabilities of blank nanoparticles in A549 cells (A) and MCF-7 cells (B) (n=6)

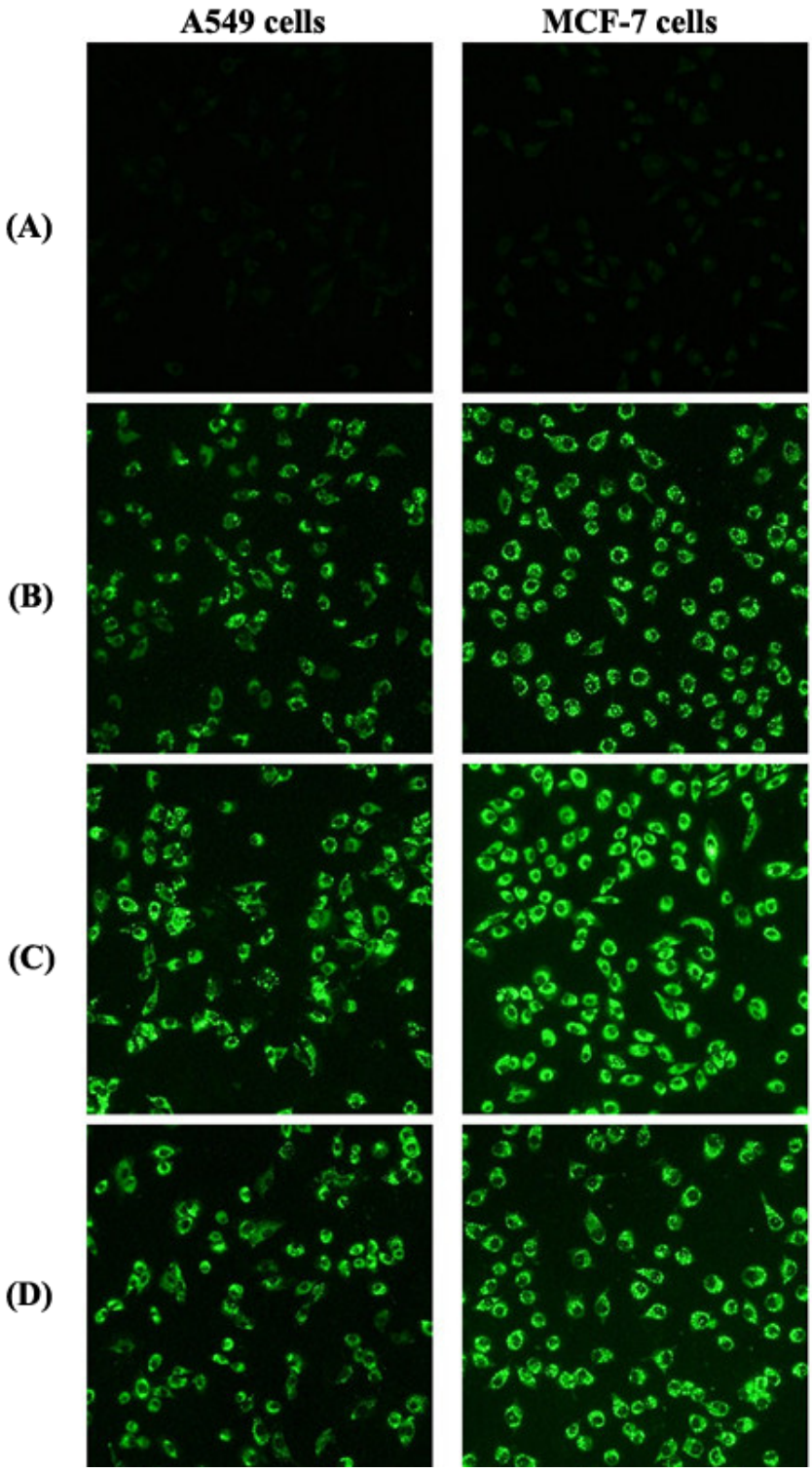

Fig. S3. Fluorescence microscopy images of A549 cells [FR(-)] and MCF-7cells [FR(+)], after incubation for 2 h treated with various formulations with an equivalent coumarin-6 concentration of 10 μg/mL. (A) free coumarin-6, (B) coumarin-6-BSANPs, (C) FA-coumarin-6-BSANPs, (D) FA-coumarin-6-BSANPs plus 2 mM free folic acid

Table S1. Preparation of BEX-BSANPs (pH 9.0; mean±S.D.;n = 3).

C_{BSA} % (w/v)	$V_{ethanol}$ (mL)	Time (h)	BEX (mg)	Diameter (nm)	EE (%)	DL (%)
1.0	7.5	0.5	4	179.0±2.1	69.64±1.24	1.58±0.11
1.5	7.5	0.5	4	174.7±3.6	74.38±1.01	1.67±0.03
2.0	7.5	0.5	4	188.2±3.3	75.92±0.92	1.65±0.06
1.5	9.0	0.5	4	172.5±2.7	78.44±0.49	1.66±0.07
1.5	12.0	0.5	4	164.9±4.0	80.15±1.06	1.78±0.05
1.5	15.0	0.5	4	159.3±2.6	73.66±0.81	1.64±0.10
1.5	12.0	1.0	4	173.6±4.9	82.56±1.19	1.73±0.04
1.5	12.0	2.0	4	168.1±1.3	77.39±0.79	1.57±0.09
1.5	12.0	1.0	6	171.4±4.7	78.12±0.45	2.61±0.16
1.5	12.0	1.0	8	180.8±4.1	62.01±1.33	2.77±0.22

Table S2. Conjugate conditions and results of FA-BSANPs. (mean±S.D.; n = 3).

Molar ratio of FA to BSA	Time (h)	Folate binding efficiency (μg FA/mg BSA)
30:1	1	15.82±2.33
50:1	1	29.25±4.26
70:1	1	35.48±3.40
90:1	1	46.31±3.71
120:1	1	48.94±5.24
90:1	4	59.84±3.51
90:1	12	74.15±2.91
90:1	24	69.65±6.65
90:1	36	75.22±3.87
90:1	48	69.17±4.23
90:1	60	70.64±3.10

Table S3. Fluorescence intensity of A549 cells [FR(-)] and MCF-7cells [FR(+)], after incubation for 2 h treated with various formulations (free coumarin-6, coumarin-6-BSANPs, FA-coumarin-6-BSANPs, FA-coumarin-6-BSANPs plus 2 mM free folic acid) with an equivalent coumarin-6 concentration of 10 μg/mL.

Samples	Fluorescence intensity	
	A549 cells	MCF-7 cells
free coumarin-6	15.74	13.16
coumarin-6-BSANPs	163.28	153.22
FA-coumarin-6-BSANPs	207.88	764.42
FA-coumarin-6-BSANPs plus 2 mM free folic acid	201.19	186.56