SUPPLEMENTARY INFORMATION

Dual-responsive nanoparticles based on oxidized pullulan and disulfide-containing poly(β -amino) ester for efficient delivery of gene and chemotherapy agent targeting to hepatoma

Hemei Wang,^{‡a} Guoyun Wan,^{‡a} Yuanyuan Liu,^{a,b} Bowei Chen,^a Hongli Chen,^c Sipei Zhang,^a Dan Wang,^a Qingqing Xiong,^b Ning Zhang^{*ab} and Yinsong Wang^{*a}

^a School of Pharmacy, Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics (Theranostics) & Research Center of Basic Medical Science, Tianjin Medical University, Tianjin 300070, China

^b Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin 300060, China

^c Research Center of Stem Cell and Biotherapy Technology, School of Life Science and Technology, Xinxiang Medical University, Xinxiang Henan 453003, China

Samples	MW ^a	PDI ^a	Oxidation degree ^b (%)
Pullulan	162808	2.571	0
oxPL-1	42785	1.386	17.58 ± 1.30
oxPL-2	29952	1.264	40.12±0.23
oxPL-3	23228	1.305	64.13±0.46

Table S1Characteristic parameters of oxPLs

^a The molecular weight (MW) relative to standard polyethylene glycol and polydispersity index (PDI) were determined by gel permeation chromatography (GPC) with a CoMetre 6000 LDI pump, a Scham-beck SFD GmbH RI2000 refractive index detector, and Shodex SB-802.5 & -804 HQ columns. The mobile phase consisted of 0.3 M NaH₂PO₄ and 1.0 M acetic acid at a flow rate of 0.5 mL min⁻¹. ^b The oxidation degrees of oxPLs were calculated from aldehyde contents determined by the hydroxyl amine hydrochloride/potentiometric titration according to the following formula.

 $Oxidation \ degree \ \% = \frac{C_{NaOH} \times \Delta V \times 161}{2 \times W_{oxPL}}$

Where C_{NaOH} was the concentration of NaOH, ΔV was the volume of NaOH consumed, and W_{oxPL} was the weight of oxPL.



Fig. S1 The ¹HNMR spectra of doxorubicin (DOX), succinic dihydrazide (SDH) and DOX-SDH conjugate.



Fig. S2 The synthesis of R-ssPBAE-oxPL-DOX (a) and the IR spectra of DOX-SDH, ssPBAE-oxPL-DOX and R-ssPBAE-oxPL-DOX (b).



Fig. S3 The viabilities of HepG2 cells treated with $poly(\beta-amino)$ ester (PBAE) and disulfide-containing $poly(\beta-amino)$ ester (ssPBAE) for 48 h.



Fig. S4 The electrophoretic mobility shift assay of branched polyethylenimine (PEI)/pDNA polycomplexes at different N/P ratios. The molecular mass of PEI was 25 kDa.



2: ssPBAE-oxPL-DOX/pDNA nanoparticles

Fig. S5 The photos of ssPBAE/pDNA and ssPBAE-oxPL-DOX/pDNA nanoparticles with the N/P ratios respectively of 9/1 and 12/1 in 10% calf serum during a 4-day storage period.



Fig. S6 The release profiles of DOX from R-ssPBAE-oxPL-DOX/pDNA nanoparticles with the N/P ratio of 12/1 in phosphate buffer salines (pH 5.5, 6.5 and 7.4).



Fig. S7 The viability (a), apoptosis (b) and cell cycle distribution (c) of HepG2 cells treated with R-ssPBAE-oxPL-DOX/pDNA nanoparticles. Q1: dead cells; Q2: late apoptotic cells; Q3: early apoptotic cells; Q4: live cells. To detect the cell apoptosis and cell cycle distribution, HepG2 cells were incubated with nanoparticles at DOX concentration of 2 μg

 mL^{-1} for 72 h.