Supporting Information

Cabral et al. 10.1073/pnas.1301348110

SI Materials and Methods

Materials. γ-Benzyl L-glutamate, and oxaliplatin were purchased from Sigma-Aldrich. Bis (trichloromethyl) carbonate (triphosgene) was purchased from Tokyo Kasei Kogyo. *N*,*N*-dimethylformamide (DMF) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Wako Pure Chemical Industries. Dichloro(1,2-diammino cyclohexane) platinum (II) was purchased from W. C. Heraeus. α-Methoxy-ω-amino-poly(ethylene glycol) (CH_3O-PEG-NH_2; molecular weight (MW), 12,000) was purchased from NOF. Alexa 648 and Alexa 488 secondary antibodies were purchased from Invitrogen. Anti-platelet endothelial cell adhesion molecule (PECAM1) was purchased from BD Pharmingen. Blocking One Buffer was purchased from Nakalai Tesque. DMEM was purchased from Sigma-Aldrich.

Mice Mating and Genotyping. Elastase 1-promoted luciferase and Simian virus 40 T and t antigens (EL1-luc/TAg) mice were mated with FVB/N mice (female; body weight, 18–20 g; age, 6 wk old), which were purchased from CLEA Japan, to generate new transgenic mice. Both EL1-luc and EL1-TAg transgenes cosegregated in subsequent generations of mice. Genotyping of the transgenic mice was done primarily by bioluminescence imaging. PCR amplification of a 1-kb luciferase fragment with primer pairs (forward, 5′-tggattctaaaacggattaccaggg-3′; reverse, 5′-ccaaaacaacaacggcggc-3′) was also done for confirmation, using DNA from the tail of mice.

Characterization of Block Copolymers. The MW distribution of PEG-*b*-poly(γ-benzyl L-glutamate) (PEG-*b*-PBLG) was determined by gel permeation chromatography (GPC) [column, TSK-gel G3000HHR, G4000HHR (Tosoh); eluent, DMF containing 10 mM LiCl; flow rate, 0.8 mL/min; detector, refractive index; temperature, 25 °C]. PEG-*b*-PBLG showed narrow MW distributions (MW/Mn: 1.09) in GPC. The degree of polymerization of PBLG in PEG-*b*-PBLG was determined to be 20 by comparing the proton ratios of methylene units in PEG (-OCH2CH2-: d = 3.7 ppm) and phenyl groups of PBLG (-CH2C6H5: d = 7.3 ppm) in ^{1}H NMR measurement.

Isolation of Tumor Cells from EL1-luc/TAg-Induced Acinar Cell Carcinomas. The cancer cell suspension was obtained by breaking the tumor tissues with 40 μm cell strainers (BD Falcon). The cells were washed twice with PBS and cultured for 12 h. Vital and adherent cells were harvested by trypsinization and PBS washed. All cells were maintained in DMEM (Sigma) containing 10% (vol/vol) FBS (Gibco) as well as 1% (vol/vol) penicillin and streptomycin (Sigma) and were cultured at 37 °C in a humidified atmosphere of 5% CO_2.

In Vitro Cytotoxicity of DACHPt-loaded micelles. The in vitro cytotoxicity of oxaliplatin and DACHPt-loaded micelles (DACHPt/m) was examined against primary EL1-luc/TAg cells. Cancer cells were plated into flat-bottomed 96-well plates at 1×10^4 per well. Cells were treated by continuous exposure to oxaliplatin or DACHPt/m in a final volume of 100 mL. Plates were incubated for 48 h at 37 °C in a humidified atmosphere with 5% CO_2, and cell viability was determined by MTT assay.

Carbohydrate-associated antigen 19-9 Expression by ELISA. The blood of the mice in the antitumor activity experiment was collected and centrifuged to obtain the serum after 28 d treatment. Carbohydrate-associated antigen 19-9 (CA19-9) expression was analyzed in a sandwich ELISA (Wuhan ElAab Science). The microtiter plate was precoated with a biotin-conjugated polyclonal antibody preparation specific for CA19-9. Avidin-conjugated horseradish peroxidase (HRP) and 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate solution were used for detection in an ELISA plate reader. CA19-9 levels were standardized with the level of EL1-luc/TAg mice of 13 wk of age. Statistical analysis was performed by Student's *t* test.