Supplementary Materials

"Sweet Tooth"-oriented SN38 Prodrug Delivery Nanoplatform for Targeted Gastric Cancer Therapy

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Supplementary Methods

Tissue Array Analysis

The tissue array (TA) and its corresponding clinical information were obtained from the Sir Run Run Shaw Hospital, Zhejiang University School of Medicine. TA contains totally 87 pairs of gastric cancer samples and corresponding paracancerous tissue (normal stomach tissue) samples. Hematoxylin and eosin (H&E) staining and immunohistochemistry staining of GLUT1 were carried out for TA. Briefly, TA was stained with GLUT1 antibody (Proteintech, 21829-1-AP, 1:2000) and incubated overnight under 4 °C for immunohistochemistry staining of GLUT1. TA was further incubated with horseradish-peroxidase–labeled goat anti-rabbit secondary antibody (Dako) for 30 min and then colored by DAB kit (Dako). The H&E and immunohistochemistry staining of TA were analyzed by Aperio XT Slide Scanner (LEICA). The expression level of GLUT1 was quantifiably determined by the histochemistry score (H-score) of TA.

Synthesis of PLA-tethered SN38 prodrug (PLA-SN38)

To a solution of PLA (n=44)-succinic acid (782.3 mg, 0.20 mmol) and SN38 (79 mg, 0.20 mmol) in 15 mL of anhydrous dichloromethane (DCM) was added 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide (EDC, 59 mg, 0.38 mmol). The reaction mixture was stirred for 5 h at 50 °C then washed with 5% citric acid, saturated $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by flash column chromatography on silica gel (DCM: MeOH = 100:1) to give PLA-SN38 prodrug (410.2 mg, 52%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.22-8.25 (d, 1H, J=9.2 Hz), δ 7.84-7.85 (d, 1H, J=2.4 Hz), δ 7.64 (s, 1H), δ 7.56-7.59 (q, 1H), δ 5.74-5.78 (d, 1H, J=16.0 Hz), δ 5.34 (s, 1H), δ 5.30 (s, 2H), δ 5.13-5.20 (m, 44H), δ 4.23-4.31 (m, 2H), δ 3.68-3.70 (t, 2H), δ 3.63-3.65 (t, 2H), δ 3.53-3.56 (q, 2H), δ 3.38 (s, 3H), δ 3.13-3.19 (q, 2H), δ 2.98-3.04 (m, 2H), δ 2.89-2.92 (m, 2H), δ 1.86-1.94 (m, 2H), δ 1.53-1.61 (m, 132H), δ 1.38-1.41 (t, 3H), δ 1.03-1.06 (t, 3H).

Analytical reverse-phase high-performance liquid chromatography (RP-HPLC) analysis of SN38 and PLA-SN38

SN38 and PLA-SN38, dissolved in dimethyl sulfoxide (DMSO), was diluted with acetonitrile/H_2O (v/v=1/1) solution and then subjected to RP-HPLC using a C18 column. Both SN38 and PLA-SN38 were monitored at 378 nm.

Determination of encapsulation efficiency (EE, %) and drug loading rate (DL, ‰)

The encapsulation efficiency and drug loading rate of SN38 were investigated by ultraviolet spectrophotometry. Briefly, lyophilized nanoparticles were dissolved in acetonitrile, then NaOH was added into the nanoparticle solution and stirred for 30 min at 37 °C to hydrolyze PLA-SN38. The suspension was centrifuged to collect the supernatant, and the SN38 content was quantitatively determined by UV-vis spectrometer (UH5300, Hitachi) at 378 nm. The encapsulation efficiency (EE, %) and drug loadings rate (DL, ‰) of SN38 in SNP and Glu-SNP were calculated according to Equations (1) and (2):

$$EE(\%) = \frac{W_{SN38\ in\ NPs}}{W_{SN38\ added}} \times 100\% \quad (1)$$

$$DL(‰) = \frac{W_{SN38\ in\ NPs}}{W_{DSPE-PEG2000+DSPE-PEG2000-Glu} + W_{PLA-SN38}} \times 1000‰ \quad (2)$$

where W_{SN38} in NPs represents SN38 encapsulated in the nanoparticles; W_{SN38} added represents SN38 added initially; $W_{DSPE\text{-}PEG_{2000}+DSPE\text{-}PEG_{2000}\text{-}Glu}$ is the total weight of matrices (DSPE-PEG_{2000} and DSPE-PEG_{2000}-Glu); $W_{PLA\text{-}SN38}$ is PLA-SN38 added initially.

Supplementary Figures and Tables

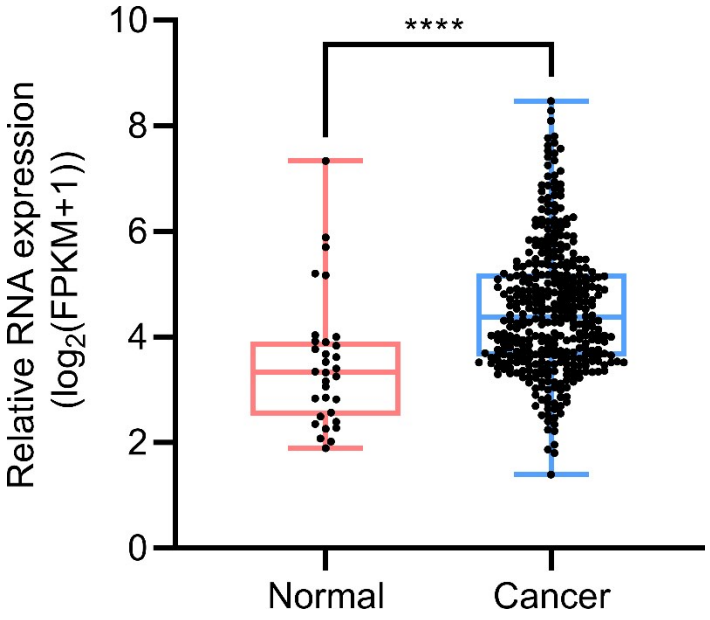

Figure S1. The RNA level of GLUT1 expression analyzed in all included gastric cancer samples (n=375) and normal gastric mucosal samples (n=32) using TCGA cohort data. *p<0.05, **p<0.01, ***p<0.001, ****p<0.001 and 'ns' represents p>0.05.

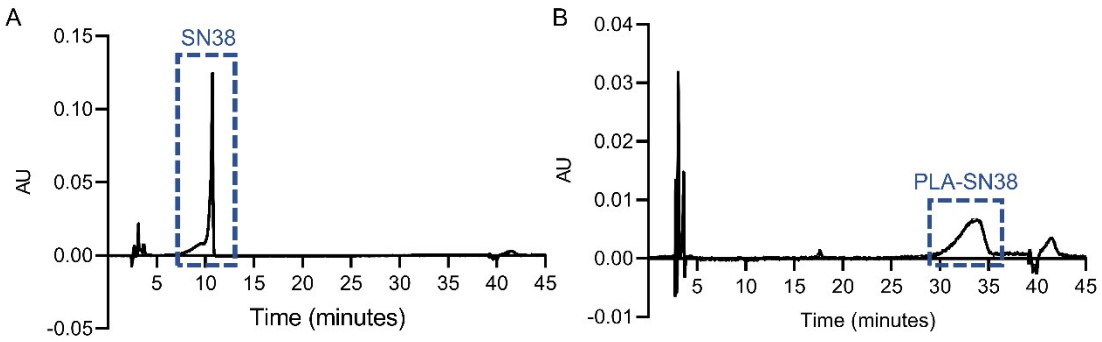

Figure S2. Representative RP-HPLC chromatogram analysis of SN38 (A) and PLA-SN38 (B).

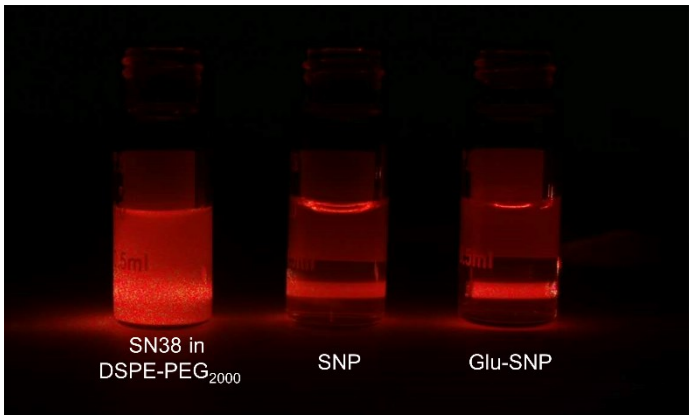

Figure S3. Tyndall effect in the solution of SN38 encapsulated in DSPE-PEG_{2000} (left), SNP (middle) and Glu-SNP (right).

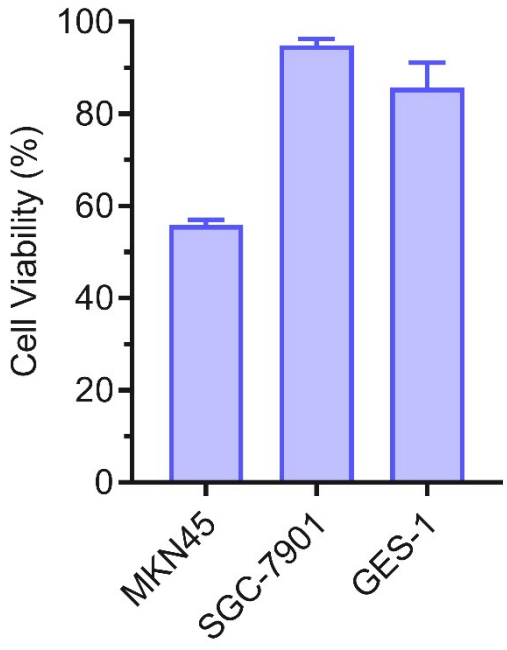

Figure S4. In vitro cytotoxicity of WZB-117. As illustrated in Figure 5A, 10 μM WZB-117 was added into medium at 12 h. Neither SNP or Glu-SNP was added at 18 h. At 42 h, the medium was replaced with the fresh medium. And the CCK-8 assay was carried at 66 h.

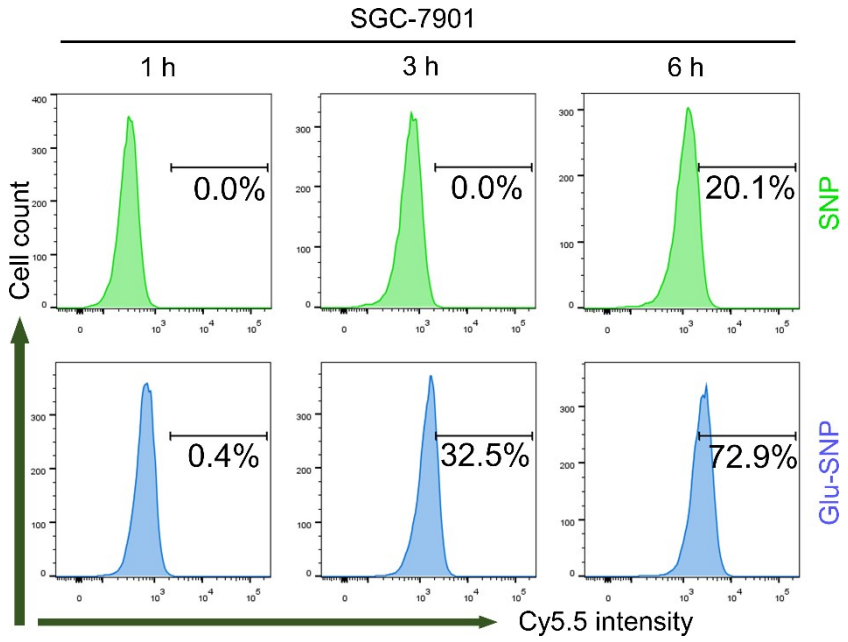

Figure S5. Representative flow cytometric analysis of Cy5.5 labeled nanoparticles (Cy5.5@SNP and Cy5.5@Glu-SNP) taken up by SGC-7901 cells for different time intervals (1 h, 3 h and 6 h) (n=3).

Table S1. Zeta Potentials, sizes, SN38 EE and DL rates of nanoparticles

NPs	D_H (nm)	Zeta Potential (mV)	PDI	EE (%) of SN38	DL (‰) of SN38
SNP	151.4±4.25	-36.7±0.30	0.096±0.027	92.3±4.2	47.1±2.14
Glu-SNP	139.2±2.41	-32.4±0.38	0.048±0.006	94.1±3.8	48.0±1.94

Table S2. Antibodies used in the Western blots.

Antibodies	Company	Product code	Dilution
Rabbit PARP1 Polyclonal Antibody	Proteintech	Cat#13371-1-AP	1:2000
Rabbit Cleaved Caspase-3	Cell Signaling Technology	Cat#9661	1:1000
Mouse anti-beta Actin	Proteintech	Cat#60008-1-Ig	1:10000
HRP-conjugated Affinipure Goat Anti-Mouse IgG(H+L)	Proteintech	Cat#SA00001-1	1:10000
HRP-conjugated Affinipure Goat Anti-Rabbit IgG(H+L)	Proteintech	Cat#SA00001-2	1:10000