Supplementary data

Reductive triblock copolymer micelles with dynamic covalent linkage deliver antimiR-21 for gastric cancer therapy

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List of Abbreviations

GPC: gel permeation chromatography
GSH: glutathione
MTT: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide
PTEN: phosphatase and tensin homolog deleted on chromosome ten
PDCD4: Programmed Cell Death Protein 4
oncomiR: oncogenic miRNA
antimiRs: antisense oligomers
PEI: polyethyleneimine
PLA: polylactic acid
PEG: polyethylene glycol
CSO: chitosan oligosaccharide
DMF: Dichloromethane, *N, N*-dimethylformamide
DMSO: dimethyl sulfoxide
THF: Tetrahydrofuran
mPEG-NH_2: poly (ethylene glycol) monomethyl ether
HATU:2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
NMM: 4-methylmorpholine
FBS: fetal bovine serum
DCM: methylene chloride
DMAP: 4-dimethylamiopryidine
RT: room temperature
NMR: Nuclear magnetic resonance
M_n: number average molecular weight
M_w: weight average molecular weight
PDI: polydispersity index
DLS: dynamic light scattering
TEM: transmission electron microscopy
CMC: critical micelle concentration
DPH: diphenylhexatriene
TBE: tris-borate-EDTA buffer
PBS: phosphate buffer saline
NP/antimiR-21: nanoparticle/miR-21
DAPI: 2-(4-amidinophenyl)-6-indolecarbamidine
RNU6B: U6 small nuclear RNA
BCA: Bicinchoninic acid
PBST**:** phosphate buffered saline with tween
NP/antimiR-NC: nanoparticle/ antimiR negative control
ANOVA: one-way analysis of variance
RES: reticulo-endothelial system
EPR: Enhanced permeation retention effect
qPCR: quantitative real-time PCR

s.c.: subcutaneous
i.v. intravenous injection
i.e. id est
PK/BD: pharmacokinetics and biodistribution

The evaluation of toxicity to kidney and inflammation induced by PEG-SS-PLA-SS-PEI copolymer *in vivo*.

To investigate whether the **PEG-SS-PLA-SS-PEI** copolymer can induce the inflammation or severe kidney toxicity, we injected the copolymer through mice tail vein and monitored dynamic changes of the relative hematology indicators at different time points (Supplementary Fig.1).

The mice were sacrificed and blood samples were collected at the time point of 1 day or 5 days after the intravenous of NP. Key parameters, such as white blood cell (WBC) and red blood cell (RBC), creatinine (CREA) and blood urea nitrogen (BUN) levels, were measured using the corresponding reagents and methods according to the manufacturers. The kidney tissue was collected and stained with hematoxilin and eosin (HE) and subsequently processed for histopathological examination under light microscope. From the results, the intravenous of copolymer did not induce the change of WBC or RBC counts, compared with the control group, during the testing period of 1 day or 5 days, indicating free from inflammation. Meanwhile, the **PEG-SS-PLA-SS-PEI** copolymers could not induce severe kidney toxicity, as the biochemical indicators (CREA and BUN) were not increased after the intravenous of copolymer and the HE analysis showed no obvious organ damage in the kidney tissue. Above all, the **PEG-SS-PLA-SS-PEI** copolymer showed low toxicity *in vivo*, promise to be a safe delivery vehicle.

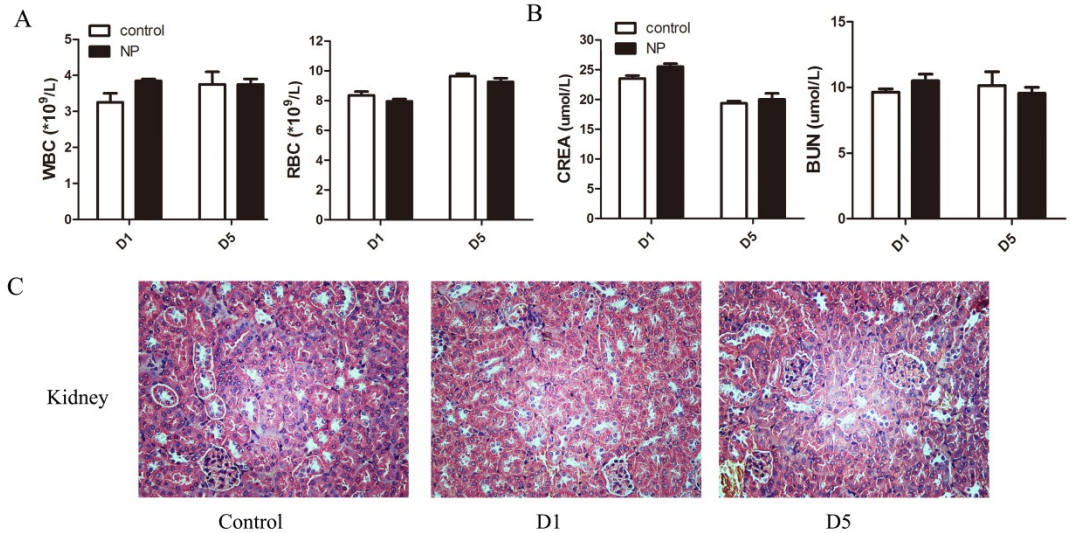

Figure S1. The evaluation of toxicity to kidneys and inflammation induced by **PEG-SS-PLA-SS-PEI** copolymer *in vivo.*

A. The intravenous of copolymers did not induce the change of WBC or RBC counts, compared with the control group, during the 1 or 5 days testing period.

B. The biochemical indicators, CREA and BUN levels in the blood samples after the copolymer micelles' treatment.
C. Pathological data (HE staining) from the kidneys of the mice (n = 5) treated with **PEG-SS-PLA-SS-PEI** copolymers. The data were collected at different time points (1 and 5 days). All hematology indicators didn't performed significant different from the control, suggesting free from inflammation response and no kidney toxicity. Errors bars reflect the standard deviation of each group (n = 5).