Blood-brain barrier–penetrating siRNA nanomedicine for Alzheimer's disease therapy

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ABSTRACT

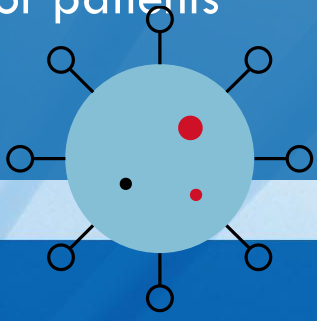
Toxic aggregated amyloid accumulation is a key pathogenic event in Alzheimer's disease (AD). Small interfering RNAs (siRNAs) show great promise for AD therapy by specific silencing of BACE1. However, lack of effective siRNA brain delivery approaches limits this strategy. Here, we developed a glycosylated siRNA nanomedicine (Gal-NP@siRNA) to target BACE1 in APP/PS1 transgenic AD mouse model. Gal-NP@siRNA exhibits superior blood stability and can efficiently penetrate the blood-brain barrier (BBB) via glycemia-controlled glucose transporter-1 (Glut1)–mediated transport, thereby ensuring that siRNAs decrease BACE1 expression and modify relative pathways. This "Trojan horse" strategy supports the utility of RNA interference therapy in neurodegenerative diseases.

BACKGROUND

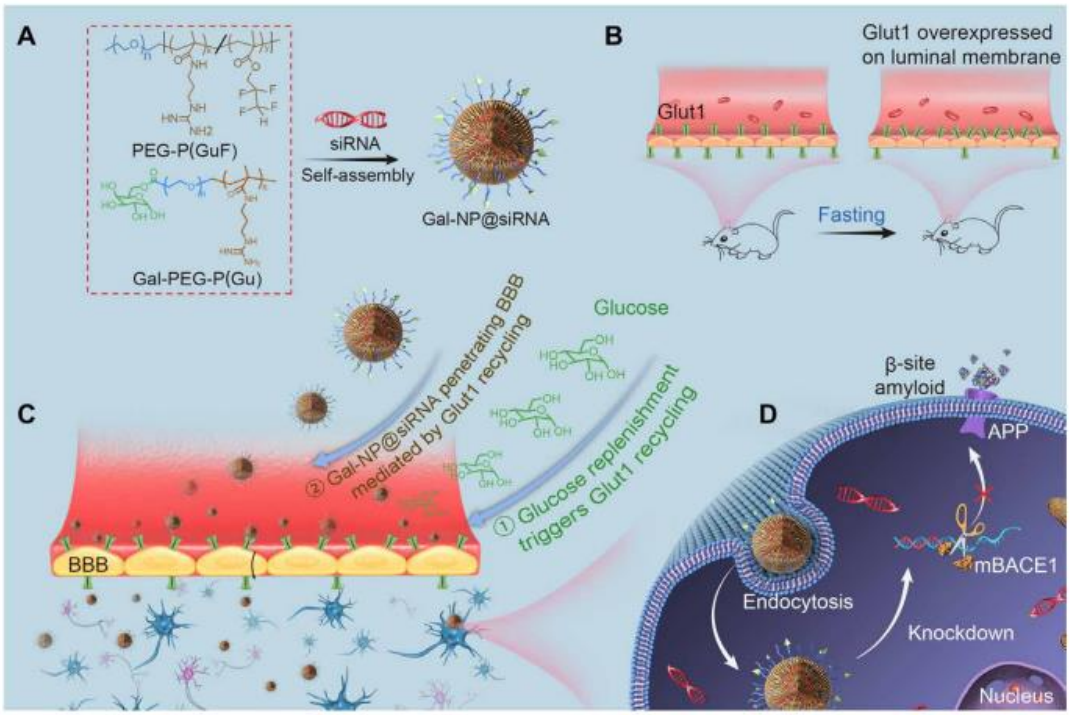
Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder, characterized by progressive deterioration of cognitive capacity (1). In 2019, AD affected more than 50 million people globally, which is expected to reach 152 million by 2050 (2). In addition, the current annual cost of AD worldwide is $1 trillion, which is estimated to double by 2030 (2). Currently, clinical therapy using acetylcholinesterase inhibitors or N-methyl-d-aspartate receptor antagonists are palliative treatment options, which only moderately improve cognition and behavior in Alzheimer's patients but do not slow disease progression (3, 4). Hence, it is imperative to develop therapeutics targeting pathological mechanisms in AD.

AIMS

This study aimed to develop a siRNA-based treatment approach for patients living with dementia.



METHODS



llustration of the formation of the glycosylated "triple-interaction" stabilized siRNA nanomedicine (Gal-NP@siRNA) and the mechanism and approach to treat AD pathology in APP/PS1 transgenic mice. (**A)** Schematic illustration of the fabrication of Gal-NP@siRNA. (**B** and **C**) Mechanism by which Gal-NP@siRNA penetrates the BBB and accumulates in the brain. Glut1 is overexpressed on the luminal membrane of the BBB after 24-hour fasting. After treatment with Gal-NP@siRNA, glucose replenishment in fasting mice results in Glut1 recycling from the luminal to the abluminal membrane of the BBB, which leads to the transport of Gal-NP@siRNA across the BBB. (**D**) Gal-NP@siRNA–mediated knockdown of BACE1 mRNA expression, which leads to reduced levels of amyloid plaques.

RESULTS

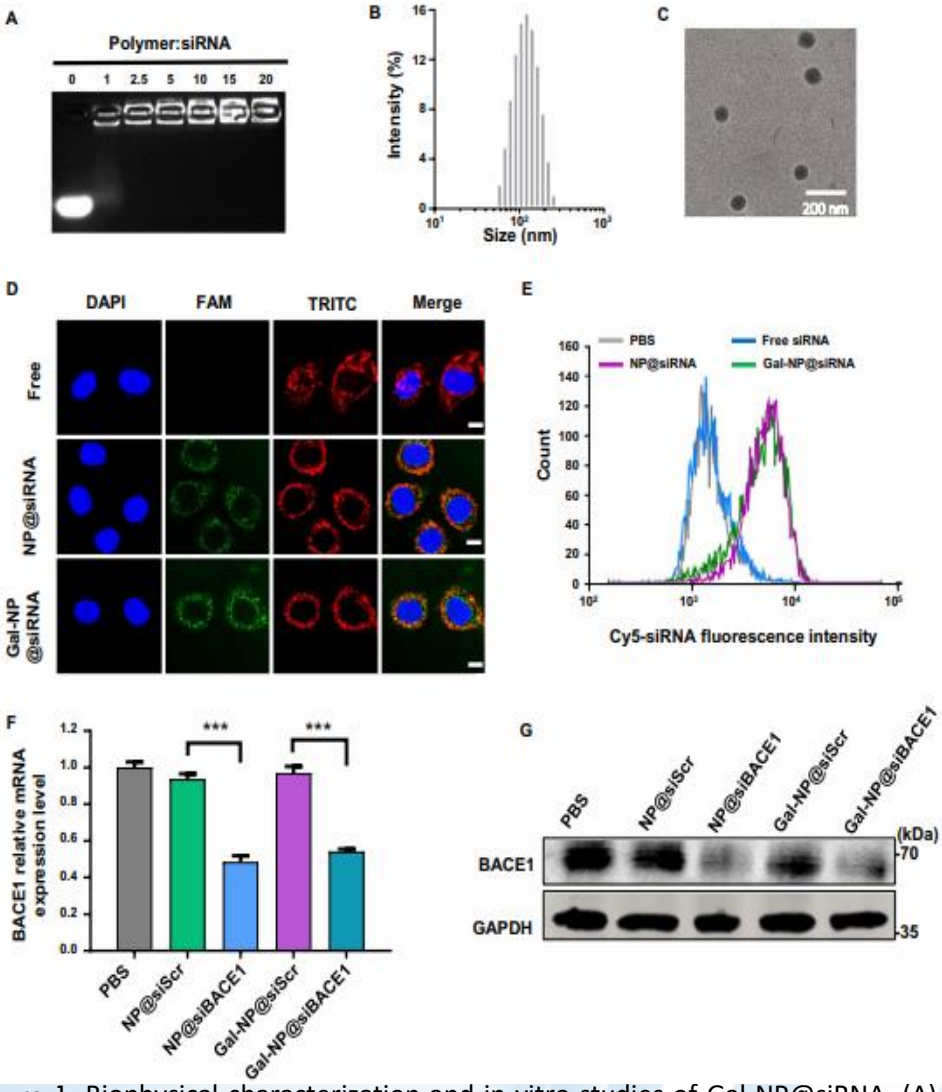


Figure 1. Biophysical characterization and in vitro studies of Gal-NP@siRNA. (A) Gel retardation assay of Gal-NP@siRNA at polymer/siRNA weight ratios of 1, 2.5, 5, 10, 15, and 20. (B) Size distribution and (C) transmission electron micrographs of Gal-NP@siRNA. (D) Confocal laser scanning microscopy images for NP cellular uptake. Images were collected for Neuro-2a cells after 4-hour NP incubation. Cell nuclei were stained with DAPI (blue), siRNA was labeled by FAM dye (green), and cell cytoskeleton was stained with TRITC-phalloidin (red) to indicate cytoplasm area. Scale bars, 10 ?m. (E) Flow cytometry analysis of Neuro-2a cells following 4-hour incubation with free Cy5-siRNA, NP@Cy5-siRNA, and Gal-NP@Cy5-siRNA. (F and G) In vitro gene silencing effects of Gal-NP@siBACE1 and controls at day 3 post transfection. BACE1 mRNA (F) and protein (G) expression levels was quantified by qRT-PCR and western blot assay, respectively. Data are presented as mean ± SEM (n = 3, ***P < 0.001).

RESULTS

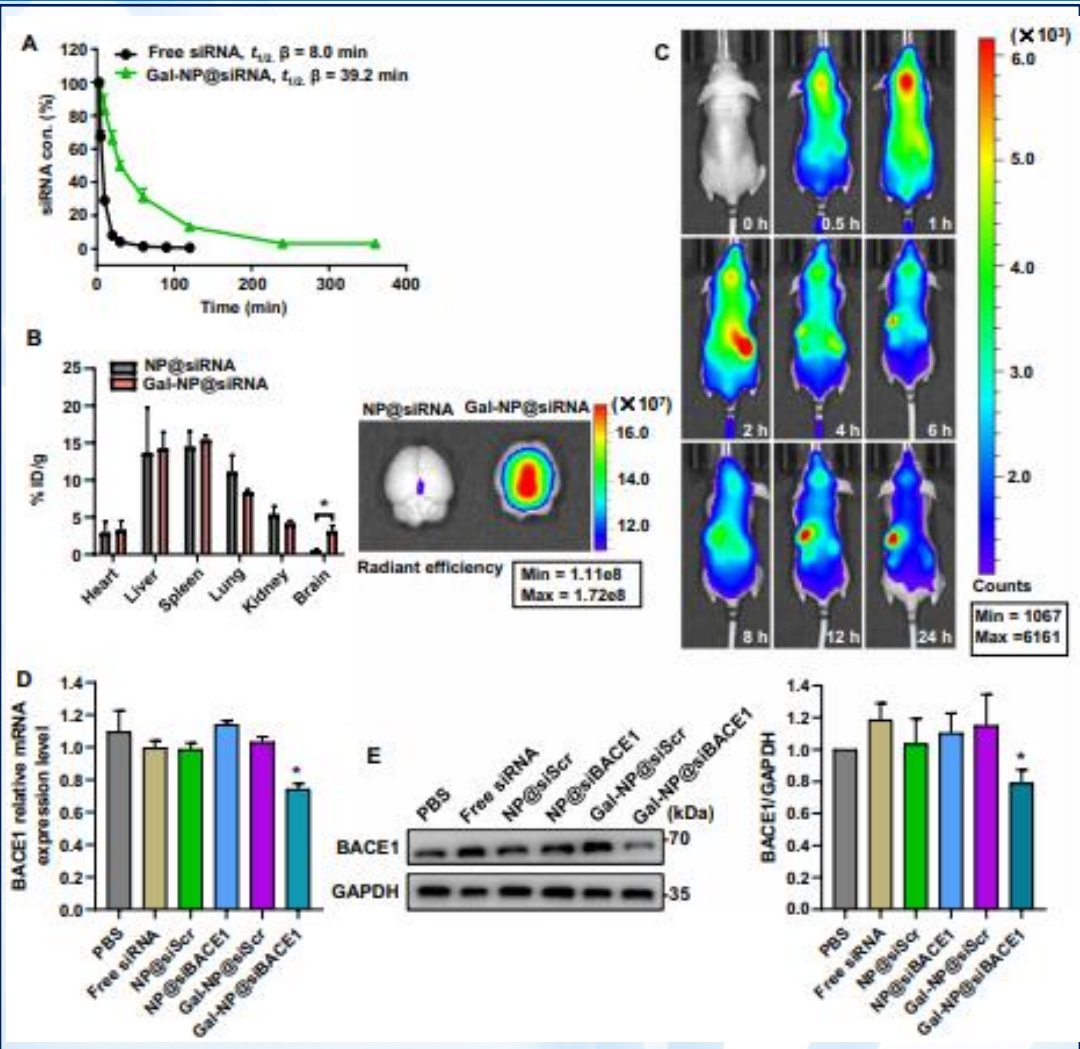


Figure 2. Fig. 3. Biodistribution and in vivo BACE1 targeting efficacy of Gal-NP@siRNA. (A) In vivo pharmacokinetics as shown by Cy5-siRNA concentration/time curves in plasma after a single-dose injection. (B) (Left) Quantification of Cy5-siRNA accumulation in different organs. Cy5-siRNA levels were determined by fluorescence spectroscopy 1 hour after tail vein injection of siRNA nanomedicine after a single-dose injection. Data are presented as mean ± SEM (n = 3, *P < 0.05). (Right) Representative image for Cy5 signal in the brain of NP@siRNA and Gal-NP@siRNA groups 1 hour after injection. (C) Time course in vivo imaging of Gal-NP@Cy5-siRNA evaluated by fluorescence imaging after a single-dose injection. (D and E) BACE1 mRNA and protein expression level in cortex was quantified by (D) qRT-PCR and (E) Western blot assay from WT mice samples, and samples were collected at day 3 after two nanomedicine treatments. Data are presented as mean ± SEM (n = 3, *P < 0.05)

RESULTS

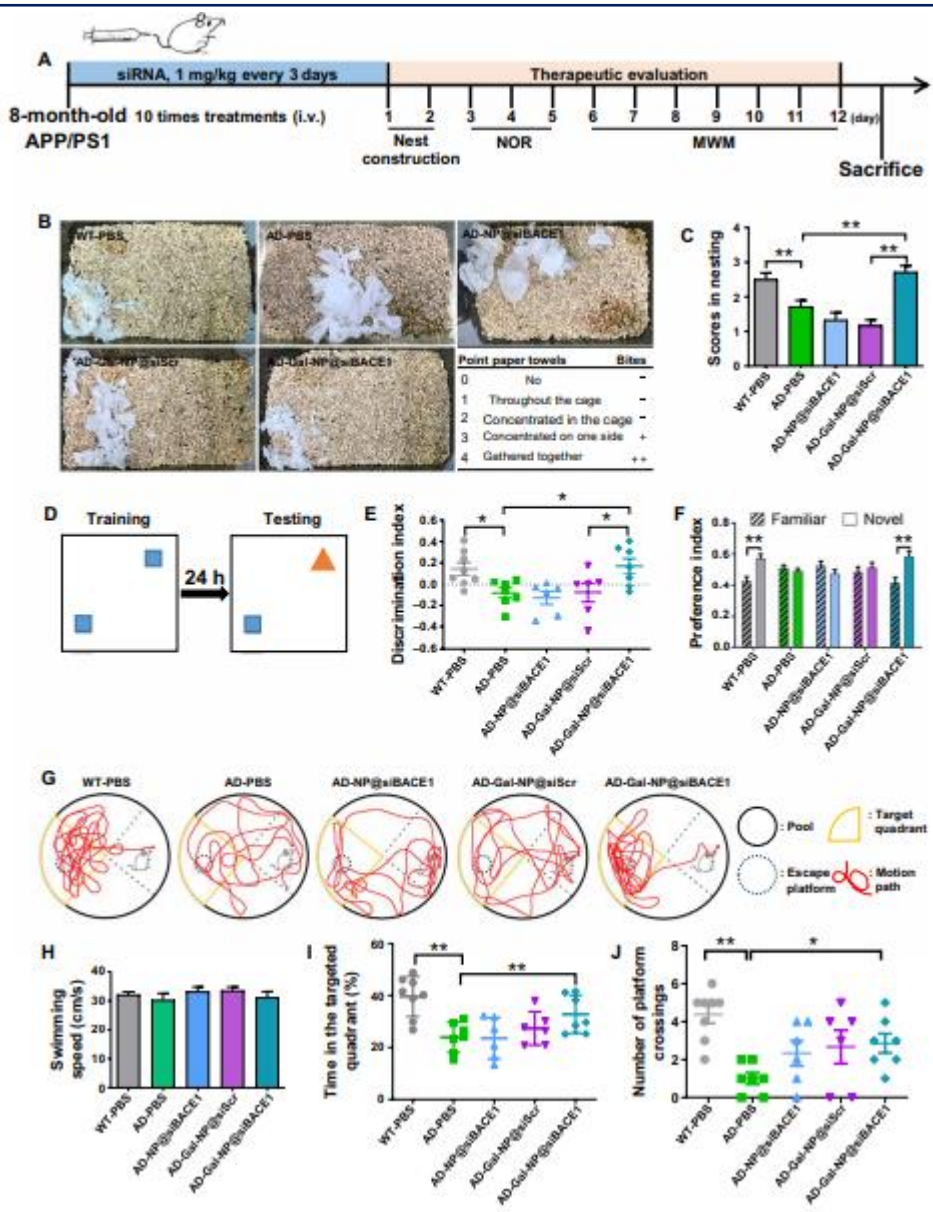


Figure. 3. Behavioral evaluation of Gal-NP@siBACE1 nanomedicine therapy in APP/PS1 mice. (A) Schematic of the experimental timeline. APP/PS1 and WT mice were treated with siRNA nanomedicine or PBS via tail vein injection every 3 days (10 cycles). Mice were then subjected to nesting, NOR, and MWM tests for memory evaluation, and samples for molecular pathological assessments were collected. (B) Representative images and scoring criteria from the nest-building experiment in APP/PS1 and control WT mice. Photos were taken 24 hours after the introduction of nesting material to the home cage. Photo credits: Yutong Zhou, Nankai University. (C) Nest-building scores for each group. (D) Setup for NOR test. (E and F) Results for NOR test. (E) DI and (F) PI of each group after nanomedicine treatment. (G to J) Data for probe test in the MWM. (G) Representative swimming track, (H) swimming speed, (I) ratio of time spent in target quadrant, and (J) number of crossing the platform location of each group on the probe test day. All behavioral test bar or plot charts are presented as mean ± SEM (n = 6 to 8, *P < 0.05, **P < 0.01)

RESULTS

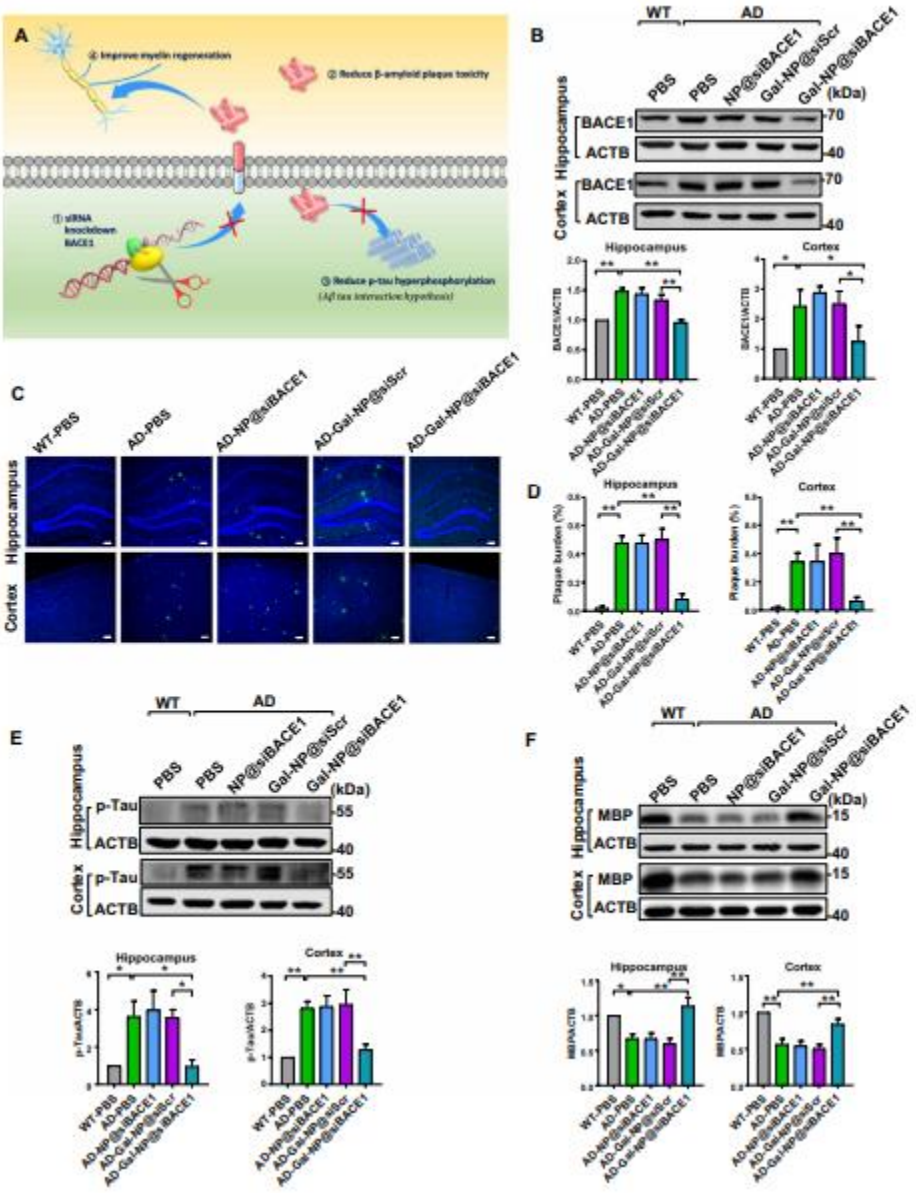
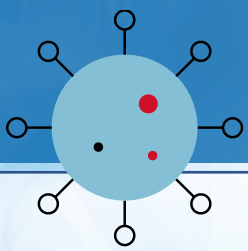


Figure.4. Therapeutic evaluation of the ability of Gal-NP@siBACE1 treatment to modulate AD hallmarks in APP/PS1 mice. (A) Mechanistic explanation for the effects of siBACE1 therapy. (B) Representative Western blot data for BACE1 protein expression in hippocampus and cortex from nanocarrier-treated APP/PS1 mice, control APP/ PS1 groups, and WT mice. Quantification of Western blotting analysis of BACE1 expression was relative to β-actin (n=3, mean with SEM, *P< 0.01. (E) p-tau and (F) MBP expression in the hippocampus and cortex for nanocarrier-treated APP/PS1 mice, control APP/PS1 groups, and WT mice (top). Quantification of Western blotting analysis was relative to β-actin (bottom) (n = 3, mean with SEM, *P < 0.05

CONCLUSION

In summary, we developed an effective strategy to deliver siBACE1 through the BBB with good circulation stability, which ameliorated AD-like pathology in APP/PS1 transgenic mice. These results indicate that our Gal-NP@siRNA nanomedicine has good clinical translation potential for AD therapy owing to ease of formulation, stability, and BBB penetration. Furthermore, our Gal-NPs could also be used to deliver siRNA in a wide range of CNS disease therapy including other neurodegenerative conditions and brain cancer.



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