Polyethylenimine-Coated Spherical Nucleic Acid Nanoparticles as Efficient, Low Dose Gene Regulatory Agents

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Statement of Purpose: Despite extensive research, developing efficient carriers to deliver small interfering RNA (siRNA) to tumor sites for therapeutic gene silencing remains an unmet challenge. Towards this goal, spherical nucleic acid nanoparticle conjugates (SNAs) consisting of 13 nm gold spheres densely coated with siRNA have recently demonstrated the ability to regulate gene expression in glioma tumors.[1] However, SNAs exhibit limited efficiency because most of the particles are sequestered in endosomes following cellular uptake and fail to reach the cytosol to silence gene expression.[2] We hypothesized that coating SNAs with a cationic polymer may improve their endosomal escape efficiency while retaining their unique architectural properties that increase siRNA stability and enable gene silencing. To test this, we developed new polyethylenimine (PEI)-coated SNAs (PEI-SNAs) and evaluated them as efficient, low-dose gene regulatory agents. We validated the gene regulation potency of PEI-SNAs by targeting eGFP in glioma cells stably expressing eGFP (U373.eGFP) as a model system. To demonstrate the therapeutic potential of PEI-SNAs, we extended their utility to target Gli1, which promotes chemotherapy resistance in glioma cells.

Methods: SNAs were prepared by coating 13 nm gold nanoparticles (AuNPs) synthesized by the Frens method[3] with thiolated siRNA and 2 kDa methoxy-polyethylene glycol-thiol, then removing unbound molecules by centrifugation. To produce PEI-SNAs, 25 kDa branched PEI was conjugated to SNAs by electrostatic adsorption, and the resulting particles were purified by centrifugation. SNAs and PEI-SNAs were characterized by transmission electron microscopy (TEM) and zeta potential measurements. To investigate the cellular uptake of SNAs and PEI-SNAs, U87 cells were treated with Cy5-labeled SNAs or PEI-SNAs for 24 hours and analyzed by flow cytometry for particle fluorescence. To investigate intracellular trafficking, U87 cells seeded on coverslips were incubated with SNAs or PEI-SNAs for 24 hours, then counterstained with LysoTracker to visualize acidic compartments, and imaged by confocal microscopy. To assess the gene silencing potency of PEI-SNAs, U373.eGFP cells or U87 cells were treated with PEI-SNAs for 48 hours, then analyzed for gene expression by flow cytometry (eGFP) and qPCR (Gli1). To validate Gli1 as a therapeutic target, we delivered Gli1 or scrambled siRNA to U87 cells with Dharmafect and assayed the cells 48-hr post transfection for sensitivity to TMZ (Alamar Blue viability assay).

Results: TEM of uranyl acetate-stained samples demonstrates a siRNA shell around the AuNP core, and the addition of PEI condenses this shell to the AuNP surface (Figure 1A). Zeta potential measurements confirm the presence of cationic PEI on the SNA surface (Figure 1B). Confocal microscopy shows that SNAs (30 nM, red) primarily co-localize with acidic compartments (green) labeled with LysoTracker, suggesting they remain trapped within endosomes or lysosomes. In contrast, PEI-SNAs (0.5 nM) do not co-localize with LysoTracker-labeled compartments, indicating they have successfully reached the cytosol (Figure 1C). Further, flow cytometry reveals that PEI-SNAs undergo drastically increased cellular uptake relative to uncoated SNAs, so they may be efficient siRNA delivery vehicles at low doses (Figure 1D). Gene silencing studies confirm this hypothesis. Using eGFP-expressing U373 glioma cells, we demonstrate that GFP-targeted PEI-SNAs can silence GFP expression by up to 50% in a dose-dependent manner (Figure 1E). We further investigated these particles in a therapeutically relevant model by targeting Gli1 in U87 GBM cells. Preliminary biological studies show that silencing Gli1 with siRNA can sensitize GBM cells to TMZ, validating our choice of target (Figure 1F). By qPCR, we demonstrate that 0.5 nM Gli1-targeting PEI-SNAs can reduce Gli1 mRNA by ~40% (Figure 1G).

Conclusions: We have developed novel PEI-coated SNAs to improve cytosolic siRNA delivery and maximize gene regulation using low nanoparticle doses. PEI-SNAs achieve enhanced cytosolic localization relative to uncoated SNAs. This translates to potent gene silencing, which we demonstrate using both a GFP-targeting model and a therapeutically relevant Gli1-targeting system. These results warrant further development of PEI-SNAs for efficient gene regulation.

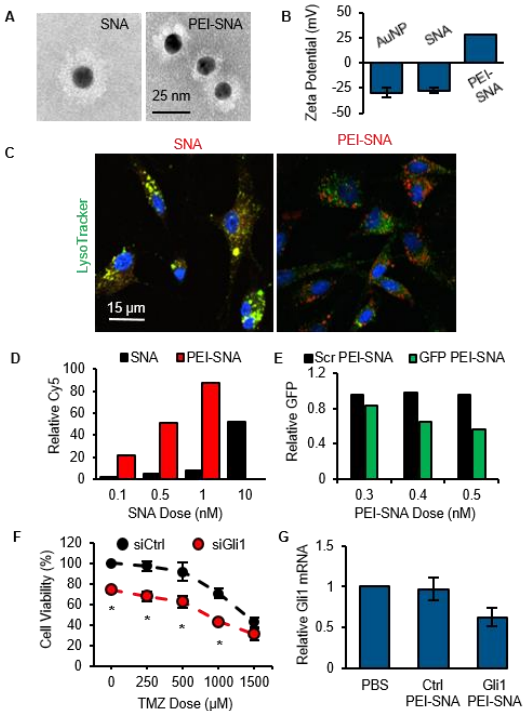

Figure 1. PEI-SNAs are potent siRNA nanocarriers.

References: [1]Jensen, Day, Ko, *et al.* Sci Transl Med. 2013; 5(209): 209ra152. [2]Wu. JACS. 2014; 136:7726-7733. [3]Frens. Nature Phys Sci. 1973;241:20-22.