Poly(beta-amino ester) nanoparticles for selective and combinatorial delivery of siRNA to brain cancer

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Statement of Purpose: Primary brain cancer is responsible for 15,000 deaths annually, and despite treatment, glioblastoma (GBM) has seen little improvement in the recent decades in its median survival time of 14 months post-diagnosis.[1] New treatment strategies are needed to help target the proliferative and migratory capacity of brain cancer cells. Short interfering RNA (siRNA) has the ability to knockdown genes responsible for tumor behavior, but safe and effective delivery strategies are necessary to use siRNA as a therapy. We describe the synthesis and optimization of novel, bioreducible poly(beta-amino ester)s (PBAEs) designed to condense siRNA into nanoparticles and release it upon entering siRNA's site of action in the cytosol. We demonstrate that these nanoparticles are safe and effective siRNA delivery vehicles, and that they selectively deliver siRNA to primary human brain cancer cells while avoiding healthy brain cells. These nanoparticles can package several types of siRNA within each nanoparticle and simultaneously knockdown each gene target.

Methods: We synthesized bioreducible monomer 2,2'-disulfanediylbis(ethane-2,1-diyl) diacrylate (BR6) following the reaction scheme shown in **Figure 1A**. BR6 could then be polymerized with previously established side chain monomer 4-amino-1-butanol (S4), and end-capped with either monomer 2-(3-aminopropylamino) ethanol (E6) or 1-(3-aminopropyl)-4-methylpiperazine (E7), to make polymers BR6-S4-E6 (R646) and BR6-S4-E7 (R647).[2] Primary human glioblastoma cells (GBM319) and primary human brain tumor initiating cells (BTIC 612) were harvested from patient intraoperative samples. Primary fetal neural progenitor cells (fNPC 34) were obtained as described previously following procedures approved by the JHU Institutional Review Board.[3] siRNA delivery efficacy was measured in primary human glioblastoma cells by delivering death positive control siRNA and comparing cell death versus cells treated with scrambled control RNA (scRNA). For knockdown of Roundabout Homolog 1 (Robo1), siRNA targeting Robo1 was purchased.

Results: We were able to synthesize polymers R646 and R647 and confirm their predicted chemical structures via H^1-NMR. A gel electrophoresis retention assay was used to confirm binding of siRNA by polymers R646 and R647. siRNA release was shown to be complete within five minutes of nanoparticle incubation with 5 mM glutathione, which mimics the cytosolic reduction potential. R646 nanoparticles containing 120 nM death positive control siRNA were delivered to GBM319 and fNPC 34 cells to compare siRNA delivery to cancer versus healthy human brain tissue. GBM319 cells exhibited 92 ± 2% siRNA-mediated cell death with 13 ± 4% toxicity, versus fNPC 34 cells which exhibited 11 ± 8% siRNA-mediated death with 18 ± 6% toxicity (**Fig. 1B**).[4] This suggests that bioreducible PBAEs preferentially transfect cancer cells versus noncancer cells. We delivered siRNA targeting Robo1 (siRobo1), a protein implicated in brain cancer migration, to BTIC 612 cells. The dose response to siRobo1 was measured by making nanoparticles in which siRobo1 and scRNA were blended in differing proportions. We can achieve near-complete knockdown of Robo1 in nanoparticles in which only 10% of the total dose was siRobo1 (**Fig. 1C**). This demonstrates that 90% of the remaining 120 nM could be used to deliver other siRNAs.

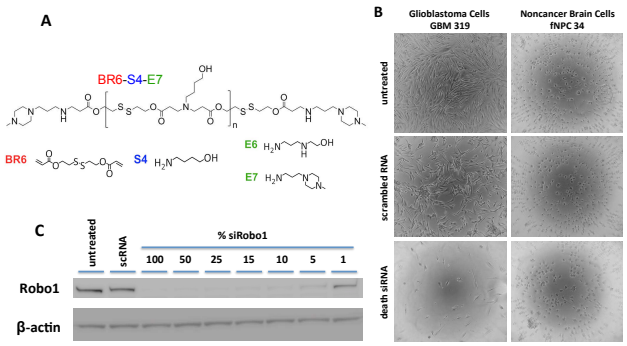

Figure 1. (**A**) Chemical structures of bioreducible PBAEs. (**B**) Delivery of death siRNA using bioreducible PBAEs shows cancer-selective delivery. (**C**) Knockdown of Robo1 is possible using low doses of siRobo1.

Conclusions: We have been able to synthesize novel, bioreducible PBAE nanoparticles that are capable of safe and effective siRNA delivery to primary human brain cancer cells. Bioreducible PBAEs encapsulate siRNA into nanoparticles, but quickly and completely release siRNA within minutes when exposed to an environment comparable to the reducing cytoplasm. We have shown that these nanoparticles can selectively deliver siRNA to human brain cancer cells while avoiding transfection of noncancer brain cells. Finally, we have been able to show that we can create nanoparticles that have the potential to deliver several types of siRNA simultaneously while achieving near-complete knockdown of each targeted gene. These results suggest that PBAEs have the potential to selectively transfect brain cancer and target multiple genes at once.

References:

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