Non-Viral Gene Therapy for Glioblastoma Multiforme

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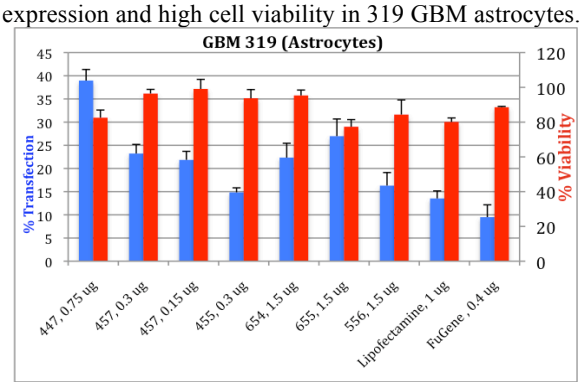
Statement of Purpose: Each year, 3 out of 100,000 in the US are diagnosed with glioblastoma multiforme (GBM), a grade IV brain cancer. Even with current treatments, median survival is only 14 months.[1] One new potential method of treatment is gene delivery. Viral gene therapy is associated with numerous health risks;[2] therefore, we used high-throughput screening of synthetic poly(β-amino esters) (PBAEs) to find materials that are both effective and safe. The end goal of the study is to deliver genes that will be expressed in GBM cells and cause apoptosis with minimal toxicity in healthy tissue. Using DNA encoding for fluorescent proteins, we have identified synthetic polymers that are suitable for this purpose. The top PBAE formulations were shown to be safer and more effective than leading commercial transfection agents like Lipofectamine 2000 and FuGene HD.

Methods: The cells used in this study were GBM astrocytes (line 319) and brain tumor stem cell (BTSC) neurospheres (551). PBAEs were synthesized as reported previously through combinatorial chemistry.[3] Polymers were diluted in aqueous solution and complexed with GFP-coding DNA at polymer-to-DNA mass ratios ranging from 30:1 to 120:1. DNA-loaded nanoparticles were added to GBM cells seeded previously in 24- or 96-well plates, using a range of doses from 0.3 ug/mL to 6 ug/mL DNA. Transfections were carried out in either culture medium containing 10% serum or serum-free medium. Particles and transfection media were replaced with normal culture medium after 2 hr. Lipofectamine 2000 or FuGene HD were used according to the manufacturers' instructions as positive controls. Particles were sized with nanoparticle tracking analysis (NTA).

After 48 hr, cells were fixed in 10% formalin, stained with 4′,6-diamidino-2-phenylindole (DAPI), and imaged by fluorescence microscopy. Transfection efficiency was defined as the number of GFP^+ cells per field normalized to the number of cells (DAPI-stained nuclei); viability was defined as the number of cells per field normalized to untreated controls. Results are expressed as mean+/-SEM.

Results: Several PBAEs tested caused high GFP expression and high cell viability in 319 GBM astrocytes.



Graph 1

Examples of successful polymers, used at polymer-to-DNA ratios from 30:1 to 120:1, are shown on Graph 1 with their associated transfection efficiency and viability. Only a few of those formulations with 80% viability or greater are shown. These and other tested polymers compared favorably to results from FuGene HD and Lipofectamine 2000 transfection. Top formulations of DNA-PBAE particles were measured by NTA to have a median hydrodynamic diameter between 140-200 nm.

The effect of serum in the transfection medium was similar to the effect of lowering dosage: viability increased while transfection decreased. These particles are expected to be used to deliver genes whose protein products are secreted and affect surrounding cells; therefore, lowering dosage to achieve 20-40% transfection will likely be sufficient for our application and will allow for low nonspecific toxicity.

Leading PBAE formulations were tested on 551 BTSC neurospheres. The BTSCs used were stably transduced with GFP; PBAE transfection was verified using DsRed DNA. Figure 1 shows 319 astrocytes transfected with GFP using PBAE 447 at 30:1 mass ratio to DNA after 48 hr days (top left) and 7 days (bottom left). GFP^+ 551 BTSCs transfected with DsRed using the same polymer formulation are shown on the right (top: GFP+DsRed; bottom: DsRed only).

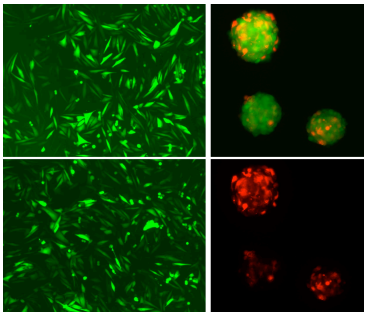


Figure 1

Conclusions: We synthesized and tested over 30 PBAEs for to identify polymers that are more effective in gene delivery than commercial agents and whose effect lasts more than a week. Emerging trends in the data will be complemented by future screenings and will allow us to tailor polymer chemistry to optimize gene delivery. We will next use these validated methods to deliver functional genes that cause apoptosis of GBM astrocytes.

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