Polymeric nanoparticles to deliver the CRISPR/Cas9 system into the human genome to treat genetic diseases

Gabriella Quiñones
Mentored by Dr. Jordan J. Green and Yuan Rui

Introduction

Poly Beta-Amino Esters (PBAEs) are a class of synthetic polymers with the potential to be used as gene therapy vectors. PBAEs have a cationic amine group, which binds with anionic nucleic acid in order to form microscopic nanoparticles. PBAEs are biodegradable, making them less cytotoxic. The CRISPR/Cas9 system is an immune response mechanism naturally found in bacteria. CRISPR, which stands for Clustered Regularly Interspaced Short Palindromic Repeats, is DNA with sections matching viral DNA. Cas9 is an enzyme that cuts DNA. CRISPR DNA is transcribed into crRNA, which binds to the Cas9 nuclease to form the CRISPR/Cas9 complex. When a bacterium is infected by a virus, the crRNA attaches to its matching viral DNA, causing the Cas9 to snip the viral DNA. Through manipulation, we can use CRISPR/Cas9 system to target specific genes that cause genetic diseases, rather than viral DNA. The goal of this project was to determine the ideal characteristics of nanoparticles for delivering the CRISPR/Cas9 system into GBM1A. Results in the GBM1A cell line were inconclusive, so research was shifted to the HEK-GFPd2 cell line.

Materials and Methods

First, the polymers were synthesized by mixing backbone monomers, sidechain monomers, and end capping monomers in different combinations to form a variety of polymers. The nanoparticles, which are a mix of nucleic acid and polymer, were made shortly before use because they degrade quickly. Weight/weight (W/W) ratio, which compares the molecular weight of the polymer to that of the nucleic acid, was varied at levels of 30, 60, and 90 w/w (Y. Rui, personal communication, August 31, 2016) to create more treatment variety. Since PBAEs and nucleic acids have opposite charges, they self-assemble into nanoparticles when combined.

To determine optimal nanoparticle formula for gene therapy, there are two stages of treatment: the initial DsRed screening, and the CRISPR transfection. In the DsRed screening (see Graph 1 and Figures 1-3), a large variety of treatments are used to transfect DsRed into cells. This stage identifies the most effective formulations. In the CRISPR transfection stage (see Graph 2), those optimal formulations are used to transfect CRISPR/Cas9, which targets the GFP genes already present in the cell line. The DsRed screening is necessary, since GFP loss could be from nanoparticle toxicity or successful transfection, whereas the presence of DsRed could only be from successful transfection (Yuen et al, 2014).

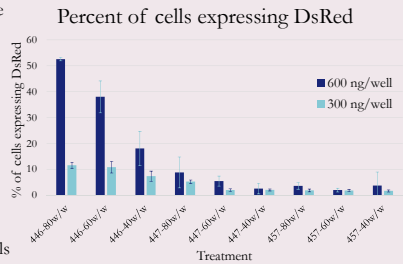
Materials and Methods (cont.)

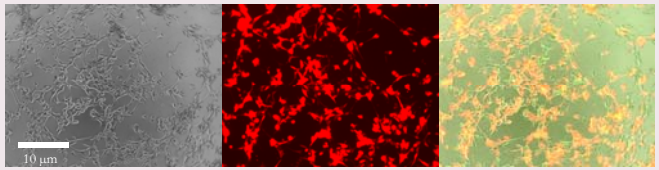
Treatment dosages of 300, 450, and 600 nanograms per well were used (Guerrero-Cázares et al, 2014). The flow cytometer was used to measure transfection efficacy by quantifying fluorescence. A higher amount of fluorescence in the DsRed screening showed the most promising treatments (Y. Rui, personal communication, August 31, 2016).

The MTT assay was used to test cell viability in the CRISPR screening. This involved administration of the MTT chemical to both treated and untreated cell samples. MTT, which is yellow, is converted by living cells into formazan, which is purple. A plate reader measured absorbance, since absorbance and living cell concentration were directly correlated. Treatments that resulted in low absorbance were determined to be toxic. The T7 endonuclease assay was used to confirm that a change to the cell genome had been made. This assay involves adding the T7 chemical to purified DNA, then running the samples through gel electrophoresis. The presence of shorter segments of DNA indicated that the DNA was cleaved by the CRISPR/Cas system, and that transfection was successful.

Results

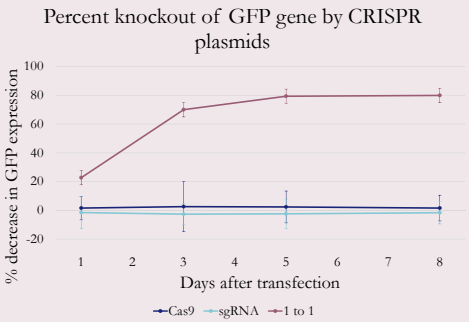
Graph 1 (right): The results of the initial DsRed screening for the HEK-GFPd2 cell line are shown here. A higher percent of fluorescing cells indicates a more effective treatment. Results show that polymer 446 at 80 w/w and 600 ng per well is the most effective treatment of the screened formulations. According to an ANOVA two-factor with replication test, the percent of cells expressing DsRed for this formulation is significantly higher than all other formulations. Each treatment has a sample size of 4, and the error bars represent standard deviation.





Figures 1-3: The bright field (left), fluorescent (center), and combined (right) microscope images of the GBM1A cell line after treatment. The nanoparticle formulation consisted of polymer 457 at 80 w/w delivered at 300 nanograms per well, which was one of the most effective formulations for this cell line.

Results (cont.)



Graph 2: The most effective treatment (446 – 80 w/w at 600 ng/well) was used to deliver the Cas9 nuclease, guide RNA, or both components to the HEK-GFPd2 cell line. GFP expression post-transfection was measured for the three treatments. Since the aim of the treatments in this experiment is to incapacitate the GFP gene, a higher decrease in GFP expression indicates a more effective treatment. This experiment also confirms that both the Cas9 and sgRNA components must be present in a treatment for gene knockout to occur. Error bars represent standard deviation. Each point represents a sample size of 8.

Conclusions

The experiments performed on the GBM1A cell line at first yielded promising results, with high transfection rates and cell viability observed especially in formulas using polymers 447 and 457, but further replication of treatments on this cell line failed to yield the same positive results as before. T7 endonuclease assays did not show cleaving of the cellular DNA, thus indicating that the loss of GFP in the treated cells had not been due to successful transfection. Therefore research was switched to the HEK-GFPd2 cell line, hypothesizing that there may have been a mutation in the GBM1A cell line. The experimentation on the HEK-GFPd2 cell line remains ongoing, but shows promising results.

References

Guerrero-Cázares, H., Tzeng, S. Y., Young, N. P., Abutaleb, A. O., Quiñones-Hinojosa, A., & Green, J. J. (2014). Biodegradable polymeric nanoparticles show high efficacy and specificity at DNA delivery to human glioblastoma in vitro and in vivo. *ACS Nano, 8*(5), 5141-5153. doi:10.1021/nn501197v

Yuen, K., Chan, C., Wong, N. M., Ho, C., Ho, T., Lei, T., . . . Jin, D. (2014). CRISPR/Cas9-mediated genome editing of Epstein-Barr virus in human cells. *Journal of General Virology, 96*(Pt_3), 626-636. doi:10.1099/jgv.0.000012