Polymeric Micelle as a Drug and Gene Delivery Carrier for Brain Tumor

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Statement of Purpose: Primary tumors centralized to the brain and spinal cord are among the most difficult to treat due to the fragile nature of the surrounding tissue. Glioblastoma (GBM) is a highly progressive cancer of CNS astrocytes that has 1.2 year median survival for the approximately 12,000 newly diagnosed patients annually[1]. The prognosis of GBM is largely due to the difficulty of treatment and the drug resistant characteristic of many of the tumor lines. Temozolomide (TMZ), a DNA alkylating drug, is commonly used to treat glioblastomas, but is rendered ineffective against the drug resistant lines by the overexpression of O-6-methlygunaine-DNA methyl transferase (MGMT), a DNA repair protein[2]. In order to overcome MGMT derived drug resistance in glioblastoma, small interfering RNAs (siRNAs) have been proposed as a precursor treatment with the goal of down regulating the overexpressed MGMT so that the Temozolomide can effectively kill the cells. In order to effectively deliver the siRNAs to the target cell, a positively charged amphiphilic copolymer (PgP) of polylactic-co-glycolic acid and polyethylenimine was designed.

Methods: PgP was synthesized and characterized by ^{1}H-NMR and GPC as previously described[3]. Monster Green Fluorescent Protein phMGFP Vector (pGFP) was used as a reporter gene to evaluate the feasibility of PGP as nucleic acid carrier in B35 Neuroblastoma and T98G Glioblastoma in both 10% Serum and Non-Serum Conditions using PgP/pGFP polyplexes with N/P ratios varying from 5/1 to 30/1. Transfection efficiency and cytotoxicity of the polyplexes was evaluated by flow cytometry and MTT assay, respectively, at 48 hours post transfection. PEI/pGFP at N/P of 5/1 was used as positive control. Followign verification of gene delivery using the pGFP an siRNA reporter (sGLO) was used to determine the optimal condtions for delivery of siRNAs to B35 cells. The transfection efficeincey and cytotoxicity were assayed 48 hours post transfection by the same means used to evaluate PgP/pGFP polymplexes. The efficacy of MGMT siRNA was asayed using real time RT-PCR 96 hours post transfection to determine MGMT protein levels in both silenced and control T98G cells after exposure to PgP/MGMT siRNA polyplexes and Lipofectamine RNAiMAX/MGMT siRNA lipoplexes. MTT assays were run in concurrently to evaluate cytotoxicity of these complexes.

Results and Discussion: PgP/pGFP polyplexes were able to successfully deliver the plasmid DNA to the target cells (B35 and T98G) shown in Figures 1 and 2. PgP was significantly more successful in delivering plasmid DNA to the cell over PEI in the more physiologically relevant 10% serum condition in both cell lines. Transfection percentage increased with increasing N/P ratio of PgP/pGFP complexes and aside from the highest N/P ratio transfections in the B35 cells, PgP shows minimal cytotoxicity despite the increasing polymer load. Transfection with PgP/siGLO polyplexes resulted in 80% of cells transfected with cytotoxicity less than 10% in the highest N/P ratio polyplexes tested.

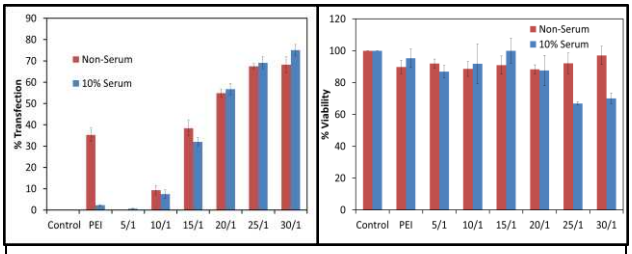

Figure 1: % Transfection and Cytotoxicity in B35 cells PEI at N/P Ratio 5/1; PgP12K/pGFP at N/P ratios shown; SEM shown, n=6

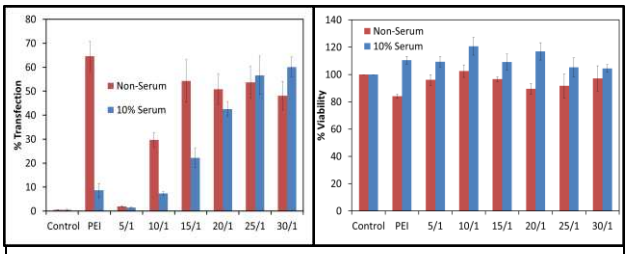

Figure 2: % Transfection and Cytoxicity in T98G cells PEI at N/P Ratio 5/1; PgP12K/pGFP at N/P ratios shown; SEM shown, n=6

Conclusions and Future Research: PgP shows promise as carrier for plasmid DNA to multiple brain cancer cell lines (B35, T98G). It shows significant improvement in transfection compared to PEI in a 10% serum condition, and shows consistent transfection across both 10% serum and non-serum conditions. Further, the siGLO experiments showed that PgP can be an effective of a carrier for siRNAs. Current research is focused on loading temozoloamide (TMZ) in PgP. In the future, we will evaluate the combinatorial therapy of TMZ loaded PgP/MGMT siRNA in the brain tumor cells in vitro.

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1. "Glioblastoma and Malignant Astrocytoma". American Brain Tumor Association (ABTA). Retrieved 16 October 2014. 2. Hegi et al. (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. The New England Journal of Medicine,352(10), 997-1003. 3. Lee et al. Trans SFB p.917 (2010).