Nanotherapeutics for Combination Drug and Gene Therapy in Treating Glioblastoma Multiforme

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Statement of Purpose: Gliomas represent approximately 80% of all malignant brain tumors, and glioblastoma multiforme (GBM), the most aggressive type, accounts for nearly half of all gliomas. Despite treatment strategies including surgery, radiation, and chemotherapy, the 5-year survival rate for brain cancer is only 35% [1]. New therapeutic strategies are necessary to improve the outcomes of this disease. Chemotherapy with DNA alkylating agents is commonly used in treatment for GBM. Research has shown that better therapeutic response of GBM tumors to alkylating agents and increased survival rate is indicative in patients with epigenetic silencing of O^6-methylguanine-DNA methyltransferase (MGMT), a gene responsible for DNA repair [2]. Therefore, we propose a treatment strategy combining drug and gene therapy to target and silence MGMT to sensitize cells to treatment with lomustine (CCNU), a DNA alkylating agent. We previously developed cationic, amphiphilic copolymer poly(lactide-co-glycolide)-g-polyethylenimine (PgP) and demonstrated its utility for nucleic acid delivery [3]. Here, we examine the ability of PgP polyplexes to overcome CCNU resistance and improve therapeutic efficacy through combination drug and gene therapy for GBM treatment.

Methods: PgP micelles were designed and synthesized for delivery of hydrophobic drugs in the PLGA core and negatively charged nucleic acids through electrostatic interactions with positively charged PEI (**Fig. 1A**). RNA binding and polyplex stability assays were performed using agarose gel electrophoresis. Cytotoxicity of PgP complexed with non-targeting siRNA was determined by MTT assay after 48 hr incubation with human glioblastoma cell line T98G. Silencing of MGMT in T98G cells on the protein and mRNA level was determined using western blotting and qPCR, respectively. For combination therapy, cell viability was analyzed 72 hrs post-treatment with PgP/siMGMT and CCNU using an MTT assay.

Results: Our results demonstrated that PgP can completely binds free siRNA and protects siRNAs from serum- and ribonuclease-mediated degradation, confirming the potential of the PgP/siRNA polyplex for *in vivo* delivery. We demonstrated that PgP/siMGMT polyplexes mediate knockdown of MGMT protein (**Fig. 1B**) as well as a significant ~56% and ~68% knockdown of MGMT mRNA (**Fig. 1C**) in T98G GBM cells compared to cells treated with PgP complexed with non-targeting siRNA (siNT) at a 60:1 and 80:1 amine:phosphate (N:P) ratio, respectively. Results from MTT assays showed that PgP/siRNA polyplexes exhibited minimal cytotoxicity compared to untreated cells when incubated with T98G human GBM cells. Further, co-treatment of PgP/siMGMT polyplexes with CCNU enhanced therapeutic efficacy in T98G GBM cells compared to treatment with the PgP/siMGMT polyplex alone or CCNU alone.

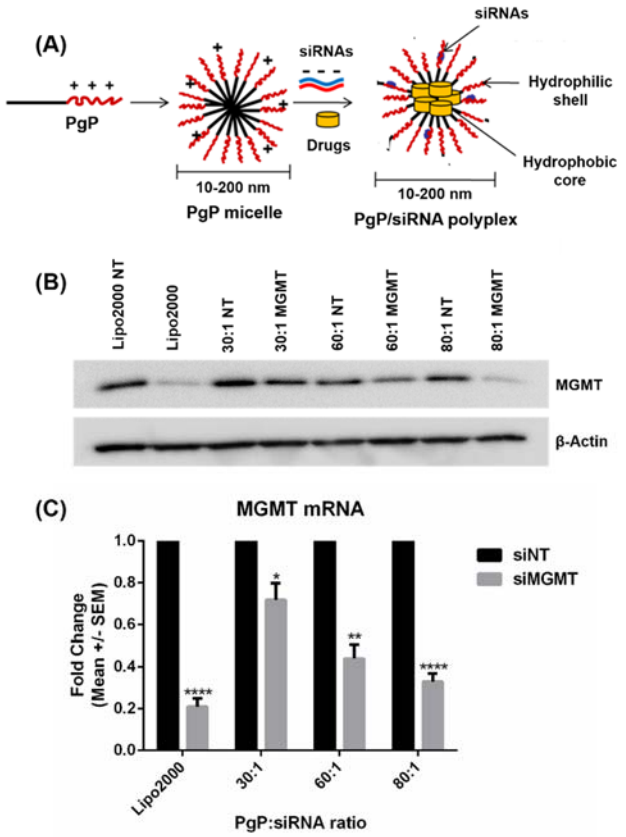

Figure 1. **(A)** Schematic of drug loaded PgP/siRNA polyplexes. **(B)** Western blot and **(C)** PCR analysis of MGMT protein and mRNA expression, respectively after treatment with PgP/siMGMT or lipofectamine 2000/siMGMT (Lipo2000, positive control). Data are mean ± SEM (N=3) compared to siNT at various N:P ratios where *P<0.05, **P<0.01, and ****P<0.0001 (t-test).

Conclusions: PgP/siMGMT polyplexes mediated significant silencing of MGMT, resulting in increased therapeutic efficacy when co-delivered with CCNU. These results demonstrate that combinatorial drug and gene therapy using PgP may overcome drug resistance and improve therapeutic outcomes. Future studies will determine efficacy of drug-loaded and siRNA-complexed PgP for combination therapy *in vitro* as well as using a xenograft GBM model for local delivery.

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