Polymer Nanoparticles for Delivery of Multiple Therapeutic Agents and their Effects on Glioma Growth

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Statement of Purpose: A significant obstacle in glioma treatment is tumor resistance to monotherapy. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles show promise for delivering anti-cancer agents including small molecules and RNA interference (RNAi). Our focus is to design polymer nanoparticles for delivering multiple therapeutic agents. Here we present multilayered nanoparticles (MLNPs) that provide a delivery system for multimodal therapy including small drugs and RNAi. Specifically, core PLGA nanoparticles containing encapsulated drugs, and having a surface polyethyleneimine (PEI) layer bound to plasmid DNA were optimized for delivering anti-cancer agents. Initially, dual plasmid delivery with MLNPs were evaluated for their effect in reducing tumor growth. These nanoparticles were further functionalized to improve transfection efficiency (TE). Camptothecin (CPT) was then encapsulated within thse particles and analyzed for potential synergistic effects with shVEGF delivery towards reducing glioma growth.

Methods: MLNPs nanoparticles were fabricated using the double emulsion protocol[1]. PEI was incorporated onto the particles as described in previous reports[2]. Plasmid DNA was electrostatically attached to surface PEI and cell penetrating peptides (CPPs) were surface conjugated. MLNPs morphology and size were determined using scanning electron microscopy (SEM), loading was quantified using established protocols[1], and zeta potential was measured using a electrophoretic light scattering. For TE optimization, luciferase plasmid was attached to MLNPs lacking CPT. Trans-1,2-cyclohexanediol (TCHD) was added to the organic phase when indicated. Human embryonic kidney 293T cells were used for TE optimization experiments. For transfection, cells were initially seeded at 15,000 cells/well, transfected 24 hours later with 1mg/mL MLNPs, and assayed for luciferase expression using a luciferin reagent assay 48 hours later. Lipofectamine was used as the positive control for transfection. For determining MLNP effect on glioma proliferation, U87 cells were cultured in the same culture medium as described above. Cells were seeded at 15,000 cells per well, and after 24hrs, exposed to 0.1 μM of CPT and an equivalent dose of CPT encapsulated MLNPs. Growth was measured for six days using Celltiter Blue proliferation assay. For our initial *in vivo* studies, A549 xenografts were established in mice and treated with MLNPs containing plasmids encoding for shHIF and shVEGF. Tumor growth was monitored for 39 days.

Results: MLNP size and loading were similar to non-modified PLGA nanoparticles. On SEM, particles were 110 - 120 nm in size and contained 8-10 μg CPT per mg PLGA. The presence of PEI on the surface can be detected by the increased surface potential on the MLNPs from 8.6 to 31.6 mV. Initially, treatment of mouse xenografts with plasmids encoding for shHIF and shVEGF resulted in significantly lower tumor volumes at 39 days (Fig1A). Particles were then optimized for TE and co-delivery with CPT instead of using dual plasmids. Surface conjugation of PLGA with PEI was effective in transfecting 293T cells. Fig 1B demonstrates that surface conjugation with CPPs, utilization of ethyl acetate (EA) instead of dichloromethane (DCM) as the organic phase, and encapsulation of TCHD further improved TE to that comparable to Lipofectamine. Optimal MLNP conjugation parameters were translated to *in vitro* cell toxicity studies using U87 glioma cells and CPT encapsulated in the PLGA core. CPT MLNPs showed similar *in vitro* cell toxicity when compared to free CPT and regular CPT PLGA nanoparticles after five days (Fig 1C). Further, blank MLNPs were able to significantly knockdown VEGF expression in U87 cells *in vitro*, and are comparable to lipofectamine (Fig 1D).

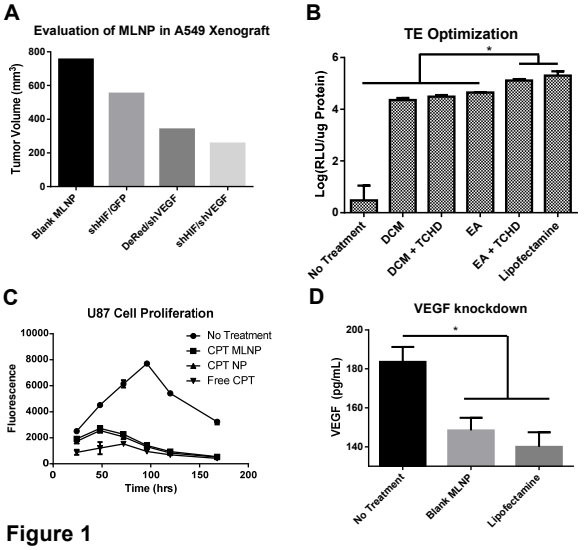

Figure 1

Conclusions: MLNPs show promise in providing a delivery vehicle for combination therapies. We hypothesized that delivering multiple therapeutic agents using MLNPs can provide improved anti-tumor effects when compared to monotherapy. Our initial *in vivo* study showed added anti-tumor effects of co-delivering shHIF and shVEGF. In addition, these particles are capable of delivering a combination of RNAi and small molecules towards reducing glioma growth *in vitro*. Future studies will focus on translating these methods for reducing tumor growth in mouse xenografts.

References:

1. Zhou J, Patel TR, Fu M, et al. *Biomaterials.* 2011.
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