Validating RNA Nanoparticles for Focused Ultrasound Delivery to Glioblastoma

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Introduction: Glioblastoma (GBM) is the most common primary brain tumor in adults with a dismal prognosis of 18-24 months following standard-of-care treatment – gross total resection, chemoradiation, and adjuvant chemotherapy with tumor-treating field therapy. The poor prognosis stems from (1) physical roadblocks to accessing the tumor microenvironment and (2) tumor heterogeneity. As such, we are investigating the administration of lipid-based nanoparticles carrying an RNA-based therapeutic payload designed to shut off key molecules that contribute to GBM proliferation and growth. We have identified a target miRNA, miR-26a, which putatively downregulates the tumor suppressor PTEN in GBM.

Hypothesis: Delivery of an antisense miR-26a-5p RNA encapsulated in a lipid nanoparticle will rescue PTEN levels in an *in vitro* model of GBM spheroid growth.

Methods: LN18 human GBM cells were cultured and transfected with either anti-miR-26a or fluorescently-tagged negative control miRNA (200 nM) encapsulated in a lipid nanoparticle, Lipofectamine 3000, then transferred to spheroid culture (FBS-free media in non-adherent plates). Spheroids were collected and processed for protein and RNA analysis. Concurrently, a microfluidic chamber was designed with the Steckl Nanolab to produce size-controlled lipid polymer hybrid (LPH) nanoparticles.

Results: Spheroid culture of LN18 cells showed a reduction of PTEN protein and mRNA level compared to LN18 adherent cells in Western blot and PCR respectively. Successful transfection of Lipofectamine:miRNA was confirmed via light microscopy which showed accumulation of Dy547-conjugated negative control miRNA in LN18 spheroids. Then, an miR-26a inhibitor was transfected in these spheroids to rescue PTEN expression. Protein analysis displayed an increased PTEN expression on Western blot in the miR-26a inhibitor group compared to the control group. With our consult, the Steckl Nanolab designed a preliminary microfluidic chamber with the potential to create nanoparticles within a 40-60 nm size range for future combination with RNA-based therapeutic payloads.

Conclusions: Spheroid culture of LN18 cells decreased PTEN expression compared to adherent LN18 cells. A spheroid approach was chosen for: (1) a more accurate mimic of the *in vivo* tumor microenvironment and (2) miR-26a is thought to be upregulated in the hypoxic spheroid environment. Following growth as spheroids, an miR-26a inhibitor can rescue PTEN expression compared to control.

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