Project Title: Ultrasound-mediated delivery of siRNA for enhancing temozolomide efficacy against glioblastoma
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Progress Report
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Project Goals: Glioblastomas are primary brain tumors that are extremely resilient against chemotherapeutics. These cancer cells exploit numerous oncogenic signal pathways that counteract the effects of cytotoxic compounds, promoting cell survival and proliferation. We have identified a signal pathway regulated by EGFRvIII, a membrane-bound protein that is only expressed by GBM cells, and have proposed arresting its expression using small interfering RNA (siRNA). The main goals of this proposal are 1) assess delivery of siRNA after FUS-mediated disruption of the blood-brain barrier (BBB), 2) assess siRNA-induced silencing of EGFRvIII expression by GBM cells implanted in rat brains, and 3) evaluate the impact of siRNA-induced silencing of EGFRvIII expression on tumor response to temozolomide.

Progress: In the first five months of the project, we have focused our efforts on examining the accumulation of nanocarriers containing siRNA in rodent brains after ultrasound-mediated disruption of the BBB. Initial studies were conducted with F98 rat glioma tumors established in the brains of Fischer rats. Tumors were sonicated with focused ultrasound (FUS) after intravenous bolus injection of Definity® and lipid-coated calcium phosphate nanoprecipitates (LCaP) containing siRNA labeled with the red fluorophore Cy5. BBB disruption was confirmed by enhanced accumulation of MRI contrast agent gadolinium administered IV within solid tumors. Harvested tumors were homogenized and analyzed for Cy5-labeled siRNA content using a spectrofluorophotometer. Compared to non-sonicated tumors, we measured 2-3 times the amount of Cy5-labeled siRNA in sonicated tumors. However, the fraction of injected siRNA that accumulated in sonicated tumors was less than 1%. In an effort to increase the amount of siRNA delivered to the tumors, we plan to use higher transmitted pressures ($P_p > 0.5$ MPa) and infuse Definity® continuously to sustain cavitation activity throughout the sonication.

Future Work: Recently we initiated a collaboration with a researcher at Dana Farber Cancer Institute who will donate patient-derived GBM cells that do and do not express EGFRvIII. Use of these cells instead of the F98 rat gliomas cells will increase the clinical relevance of this project dramatically as the dependence of the growth and survival of human GBM cells on EGFRvIII signaling is well documented. This requires switching from rats to mice for establishing the tumor model. Mice are much smaller than rats and thus an added benefit will be a significant reduction in the amount of siRNA required, leading to significant cost savings. As a preliminary step, we have tested delivery of LCaP containing Cy5-labeled siRNA to murine brain using the same acoustic parameters from the rat experiments. Comparable levels of Cy5-labeled siRNA were measured in murine brain after FUS-mediated BBB disruption. We will use the refined acoustic parameters in subsequent studies. Provided the fraction of LCaP containing siRNA remains low, we will link a GBM-specific targeting ligand, chlorotoxin, to the carrier surface. We anticipate completing this part of the project in February and moving forward with evaluating changes in target protein expression.