Fibrin-Binding, Peptide Amphiphile Micelles for Targeting Glioblastoma

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Statement of Purpose: Glioblastoma multiforme (GBM), a grade IV astrocytoma, accounts for 70% of all malignant gliomas and carries a 5-year survival rate of less than 5%[1]. Treatment of glioblastoma represents one of the most formidable challenges in oncology due to the inability to completely surgically resect the tumor due to a diffuse invasion of the surrounding normal brain tissue. Consequently, post-surgical chemotherapy regimens are essential for the treatment of gliomas, but therapeutic advancement has remained disappointing due to poor penetration across the blood-brain-barrier (BBB). Recent developments in micelle technology have yielded promising results in the delivery of therapeutic agents and through surface-modifications with ligands, micelles can bind to markers that are selectively expressed on cancer cells or the tumor vasculature. Since fibrin deposition within and around the tumor area is characteristic to brain tumors, we examined the targeting capabilities of peptide amphiphile micelle nanoparticles functionalized with the clot-binding peptide, cysteine-arginine-glutamic acid-lysine-alanine (CREKA). Earlier studies confirmed CREKA can home to sites of fibrin found on atherosclerotic plaques and target breast cancer[2,3]. In this study, the potential of CREKA-micelles as a targeting nanoparticle system for gliomas via active binding to fibrin within the tumor blood vessels was investigated.

Methods: *CREKA micelle synthesis and characterization*: The [Cys-Arg-Glu-Lys-Ala] peptide was synthesized using Fmoc-mediated solid phase peptide synthesis methods on an automated peptide synthesizer (Protein Technologies). Peptides were cleaved, deprotected, and purified by reverse-phase HPLC. Cysteine-containing peptides were conjugated via a thioether linkage to DSPE-PEG(2000)-maleimide. Cy7 mono-N-hydroxysuccinimide ester was conjugated via a peptide bond to DSPE-PEG(2000)-amine. CREKA micelles were assembled by mixing the Cy7 and peptide-containing DSPE-PEG(2000) (10:90 molar ratio) amphiphiles in water or PBS. Non-targeting micelles were assembled by combing DSPE-PEG(2000)-Cy7 and DSPE-PEG(2000)-maleimide. The critical micelle concentration (CMC) was determined using the 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence assay and dynamic light scattering (DLS) and transmission electron microscopy (TEM) was used to determine micelle size.

Animal experiments: To assess the targeting abilities of Cy7- and Cy7-CREKA-micelles, 8-10 weeks old male C57BL/6 mice were intracranially injected with $4x10^5$ GL261 murine glioma cells suspended in 5 µl PBS into the right hemisphere of the brain. Tumors were allowed to grow for 1 week before administration of 100 µl of 1 mM Cy7-CREKA-micelles, Cy7-micelles, or PBS via tail vein injection. *In vivo* images at 1, 2, and 3 hours post-injection were obtained using a Xenogen IVIS 200 imaging system (Caliper Life Sciences). Mice were then euthanized and organs (e.g. brain, heart, lungs, liver, kidneys, spleen, large intestines, and bladder) were excised at 3 hours, 24 hours, and 7 days post-injection and imaged *ex vivo*. Quantification and comparisons of the fluorescence signal was achieved via Living Image software (Perkin Elmer). Following optical imaging, the brain, liver, and kidney were immediately frozen and tissue sections were stained with H & E. Brain sections were also processed for immunohistochemistry using an antibody against fibrinogen.

Results: The CMC of micelles was determined to be $9.3x10^{-7}$ µM and conjugation of the CREKA peptide to Cy7-micelles increased the average particle size from 7.6 ±1.2 to 8.2 ± 1.2 nm. Upon intravenous administration to GL261 glioma bearing mice, Cy7-micelles passively accumulated at the brain tumor site via the enhanced permeability and retention (EPR) effect, and Cy7-CREKA-micelles displayed enhanced tumor homing via active targeting as early as 1 hour after administration (Figure 1), as confirmed via *in vivo* and *ex vivo* imaging and immunohistochemistry. Biodistribution of micelles showed an accumulation within the liver and kidneys, leading to micelle elimination via renal clearance and the reticuloendothelial system (RES). Histological evaluation showed no signs of cytotoxicity or tissue damage at all time points.

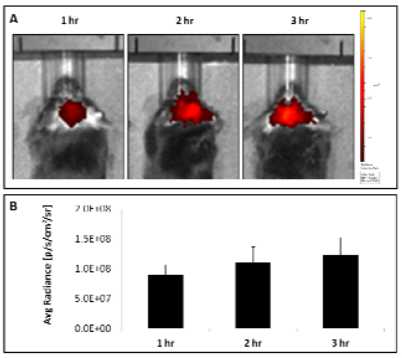

Figure 1. Cy7-CREKA micelles accumulate in brain tumors as early as 1 hr post-injection.

Conclusions: Our study demonstrates a new avenue for targeting glioblastoma via fibrin-targeting, while verifying the biocompatibility and safety of Cy7-CREKA micelles. Future studies will harness these capabilities to achieve highly efficient, chemotherapeutic drug delivery for the treatment of malignant gliomas.

References:

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