Developing a platform technology to cross the blood brain barrier and deliver drugs to specific populations

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Introduction

Prognosis for breast cancer patients diagnosed with brain metastases is poor, with survival time measured merely in months. This can largely be attributed to the limited treatment options capable of reaching the tumour as a result of the highly restrictive blood-brain barrier. While methods of overcoming this barrier have been developed and employed with current treatment options, the majority are highly invasive and non-specific, leading to severe neurotoxic side effects. A novel approach to address these issues is the development of therapeutics targeting receptor mediated transport mechanisms on the blood-brain barrier endothelial cell membranes. Using this approach, we intercalated doxorubicin into a bifunctional aptamer targeting the transferrin receptor on the blood brain barrier and epithelial cell adhesion molecule on the metastatic cancer cells. The ability of the doxorubicin loaded aptamer to transcytose the blood brain barrier and selectively deliver the payload to epithelial cell adhesion molecule-positive tumours was evaluated in an *in vitro* model and confirmed for the first time *in vivo* using the MDA-MB-231 breast cancer metastasis model (MDA-MB-231Br). We show that co-localised aptamer and doxorubicin are clearly detectable within the brain lesions 75 minutes post administration. Collectively, the results from this study demonstrate that through intercalation of a cytotoxic drug into the bifunctional aptamer, a therapeutic delivery vehicle can be developed for the specific targeting of epithelial cell adhesion molecule-positive brain metastases.

Aim

This project **aims** to generate a bi-functional aptamer capable of transcytosing the blood-brain-barrier and specifically deliver cytotoxic payloads to metastatic brain tumours.

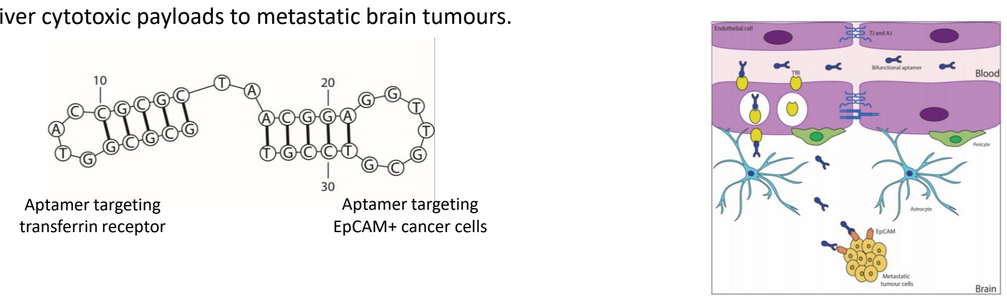

Figure 1: Schematic of bifunctional aptamer structure and figurative representation of the bifunctional aptamer transcytosing the blood-brain-barrier and targeting metastatic tumour cells.

Methods and Results

Targeted drug delivery enhances efficacy while being protective to healthy cells

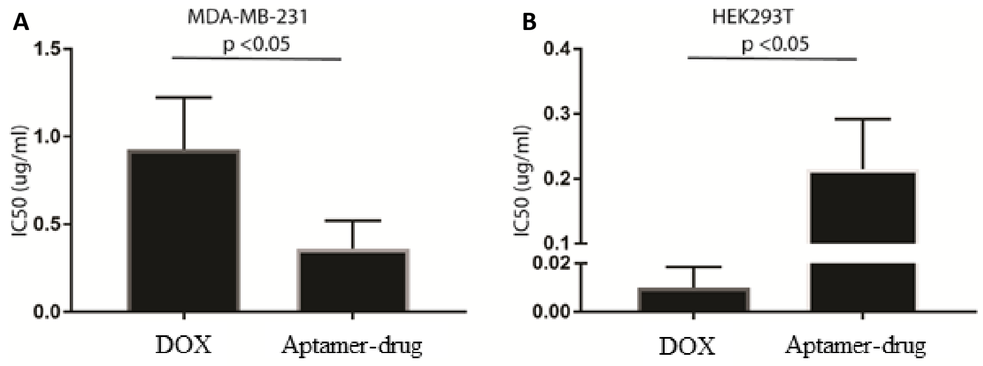

Figure 2: Assessment of drug (DOX) and aptamer-drug cytotoxicity. Following 48 h of treatment of drug and aptamer-drug (0 to 40µg/mL), cell viability was assessed and the IC50 value for each treatment was calculated. Data representative of three independent experiments. (n=3).

The bifunctional aptamers can cross the blood brain barrier in a healthy animal model and an animal model of breast cancer brain metastases

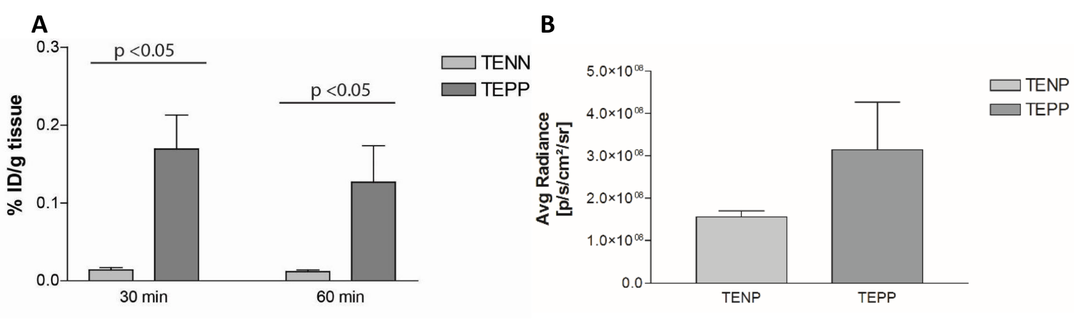

Figure 3. A: Biodistribution of aptamers in the brain in a healthy animal model; and B: Biodistribution of aptamers in the brain in an animal model of metastatic breast cancer. TENP: negative control aptamer; TENN: negative control aptamer; TEPP: positive control aptamer.

Drug attachment stabilises the aptamer structure and increases drug dose in the brain

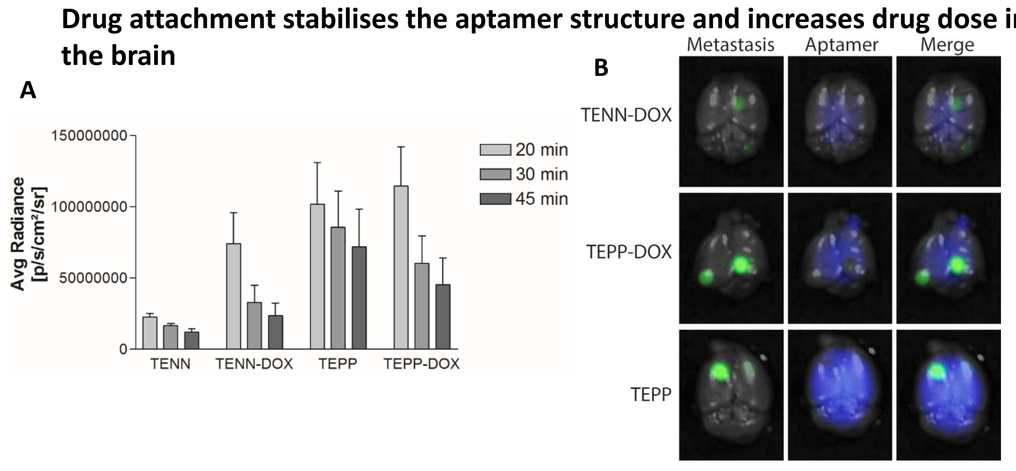

Figure 4. A: Biodistribution of aptamers and aptamer-drug in the brain of an animal model of metastatic breast cancer; and B: Biodistribution of aptamer and aptamer-drug in the brain of an animal model of metastatic breast cancer. Green signal represents cancer cells and blue/purple represents aptamer. TEPP signal was determined at 45 minutes while TENN-DOX and TEPP-DOX signal was determined at 75 minutes. TENN: Negative control aptamer; TENN-DOX: Negative control aptamer-drug; TEPP: Positive control aptamer; TEPP-DOX: positive control aptamer-drug.

The bifunctional aptamer deliver drugs across the blood brain barrier and delivers drugs only to tumour cells

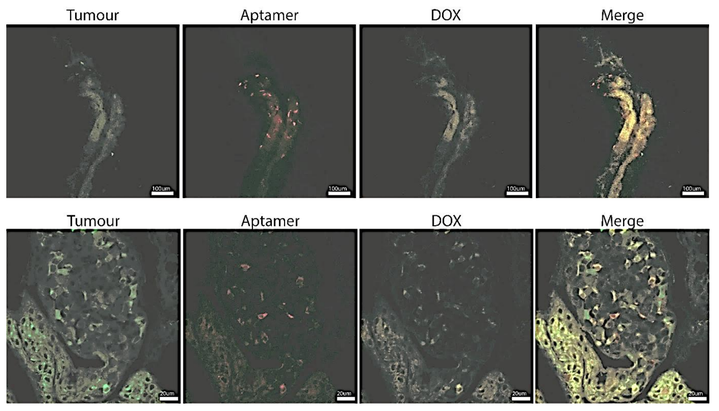

Figure 5. Brain distribution of aptamer-DOX conjugates following tail vein injection. Brains were excised 75 minutes post intravenous administration and imaged using laser scanning confocal microscopy Green: GFP tumour; red: bifunctional aptamer; and yellow: DOX. Scale bar: A: 100 µM and B: 20 µM

The bifunctional aptamer reduces tumour burden in the brain

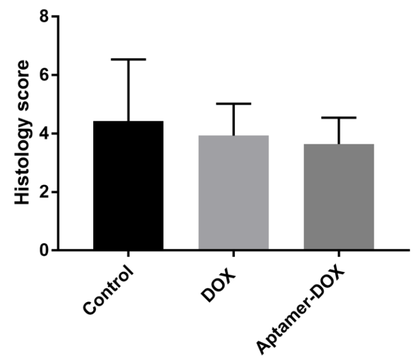

Figure 6: Animals were dosed four times over a period of two weeks with either control, free drug, or aptamer-drug. At the end of two weeks treatment, the brains and organs were excised. The brains were cryosectioned and the amount of tumour was assessed quantitatively. Tumour burden was assessed in brain sections of mice treated with control, free drug (DOX), or aptamer-drug

The bifunctional aptamer decreased systemic tumour burden

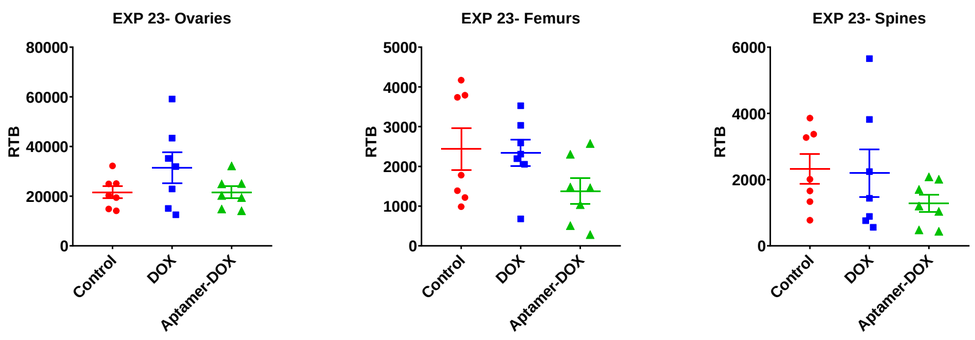

Figure 7. Organs and bones showing metastatic burden were excised for accurate quantitation of metastatic burden by genomic qPCR quantitation of relative tumour burden by detection of a marker gene (neomycin resistance) present only in tumour cells

Conclusions and future work

The effective treatment of brain metastases is greatly hindered because of their location. While aptamers targeting EpCAM positive solid malignancies from the neck down have been developed, there still remains no aptamer capable of crossing the BBB and specifically targeting EpCAM positive brain metastases. Through the fusion of two mono-functional aptamer sequences we have generated a bifunctional aptamer capable of crossing the BBB. Using flow cytometry and confocal microscopy it was confirmed that the conjugation of the EpCAM and transferrin aptamers had no effect on specificity and selectivity. Using an in vitro BBB model we confirmed the ability of the bifunctional aptamer to transcytose the BBB and specifically target EpCAM positive cells. Furthermore, the ability of this bi-functional aptamer to transcytose through the BBB was confirmed using a healthy animal model and an animal model of breast cancer brain metastases. Using a metastatic TNBC mouse model we demonstrated that a higher amount of the aptamer-drug was measured in the brain. In a small treatment trial, we have also demonstrated efficacy both in reducing tumour burden in the brain as well as systemically. These results demonstrate the potential this aptamer has for the specific targeting and treatment of brain metastases. we have demonstrated that the aptamer-drug enters the brain specifically and only enters cancer cells in the brain, thus mitigating any toxic effects on healthy brain tissue. A drug dose equivalent to 2.5 times the IC_{50} decreased tumour burden in the brain. These results are promising and suggest that investigating increased dosing as well as treating tumour burden in other organs concurrently with treating brain metastases will effectively reduce brain metastases will mitigating any toxic side effects. We are now investigating this platform technology against primary brain cancers with different aptamers targeting other cell surface receptors.

References: 1. Walbert T, Gilbert MR. The role of chemotherapy in the treatment of patients with brain metastases from solid tumors. International Journal of Clinical Oncology. 2009;14(4):299-306. 2. Fortin D. The blood-brain barrier: its influence in the treatment of brain tumors metastases. Current Cancer Drug Targets. 2012;12(3):247-59. 3. Tseng, L. M. *et al.* Distant metastasis in triple-negative breast cancer. *Neoplasma* 60, 290-294 (2013). 4. Gabathuler, R. Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. *Neurobiology of Disease* 37, 48-57 (2010). 5. Daniels, T. R. *et al.* The transferrin receptor and the targeted delivery of therapeutic agents against cancer. *Biochimica et Biophysica Acta* 1820, 219-317 (2012). 6.Yu YJ, Zhang Y, Kenrick M, Hoyte K, Luk W, Lu Y, et al. Boosting brain uptake of a therapeutic antibody by reducing its affinity for a transcytosis target. Science Translational Medicine. 2011;3(84).

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