Multimodality cell tracking of triple negative breast cancer via the Organic Anion-Transporting Polypeptide 1 (OATP1) gene

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Introduction: Globally, breast cancer is the most common cancer diagnosis among women as well as the leading cause of cancer-related death in women1. Due to the dynamic nature of the tumour, the pathogenesis of breast cancer remains poorly understood. Reporter genes are a valuable resource as they encode detectable products, allowing for quantitative “reporting” of cells and cell products. Bioluminescence imaging (BLI), based on the luciferase gene, exhibits high sensitivity, enabling detection of small numbers of molecular events in vivo. However, BLI suffers from low spatial resolution and lack of 3-dimensional information. Multimodality imaging can mitigate issues inherent in a single modality; magnetic resonance imaging (MRI) can complement BLI, but the sensitivity provided by MRI reporter genes developed to date is suboptimal2.

Rationale: A rat-derived protein belonging to the Organic Anion Transporting Polypeptide 1 (OATP1) family, called OATP1A1, was shown to increase luciferin uptake and enhance BLI signal from luciferase-expressing cells3. OATP1A1-expressing cells were also shown to take up a paramagnetic agent capable of generating T₁-weighted MRI contrast called gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)4. Our objective is to extend this toolbox by comparing image contrast from expression of other OATP1 members, namely the human OATP1B1 and 1B3 orthologs. We hypothesize that breast cancer cells engineered to express luciferase and oapt1 genes will exhibit increased R₁ relaxation rates in Gd-EOB-DTPA-enhanced MRI at 3 Tesla (T), while concomitantly displaying enhanced BLI signal generation.

Materials and Methods: Human metastatic breast cancer (MDA-MB-231) cells were engineered via lentiviral transduction to express tdTomato Luciferase and zsGreen along with either rat OATP1A1, human OATP1B1, or 1B3. The cells were subsequently sorted via flow cytometry and immunofluorescence stains were produced to confirm protein expression. Cells were treated with 0.5 mM Gd-EOB-DTPA for 90 minutes and washed 3× with phosphate buffered saline prior to imaging. R₁ relaxation rates of cell pellets were acquired using an Inversion Recovery Fast Spin Echo pulse sequence with TI = 50, 60, 70, 75, 80, 100, 150, 250, 350, 500, 750, 1000, 1250, 1500, 2000, 2500, 3000 ms; TR = 15 000 ms; TE = 19.1 ms; and ETL = 4. A trypan blue assay was used to evaluate viability following 0.5 mM Gd-EOB-DTPA treatment for 90 minutes.

Results: No difference in cell viability was found between treated and untreated control and OATP1-expressing cells (p>0.05). Pilot in vitro data showed that, following Gd-EOB-DTPA treatment, OATP1A1- and 1B3-expressing cells had elevated R₁ relaxation rates (3.91 ± 0.44, 3.39 ± 0.22 s⁻¹) relative to 1B1-expressing cells (1.43 ± 0.07 s⁻¹) and treated control cells (0.792 ± 0.03 s⁻¹) at 3T (Figure 1). Furthermore, there was no observed difference in R₂ relaxation rate between treated controls, untreated OATP1-expressing cells, and untreated controls.

Discussion: Currently, breast cancer is largely studied in vitro, using simple research models to assess complex characteristics. In recent years, MRI reporter gene development has focused on iron-based mechanisms, which interfere with iron homeostasis and cell phenotype, thereby confounding results and virtually eradicating their potential for human application5. The OATP1 system could shift the breast cancer research model in a new direction, and, in the larger scheme of things, fill a vacuum of information not provided by other modalities. Following in vitro characterization, we hope to assess in vivo sensitivity of our system in mouse models. If successful, this new technology could enable dynamic tracking of breast cancer cells and cell products in their natural environments with combined high spatial resolution, sensitivity and 3D information.