breast cancer. The first consists of a HIF-1α-CRE-ER\textsuperscript{TAM} ‘hypoxia sensing’ construct coupled with a loxp DsRed loxp DTR-EGFP cassette (Diphtheria Toxin Receptor: DTR). Under normal conditions the HIF-1α-CRE-ER\textsuperscript{TAM} fusion protein is expressed, but immediately degraded, but under hypoxia HIF-1α degradation is inhibited leading to accumulation. 4-OHT Administration translocates HIF-1α-CRE-ER\textsuperscript{TAM} to the nucleus mediating loxp recombination in the coupled cassette. This allows lineage tracing of hypoxic cells and their progeny and selective ablation through the DTR using Diphtheria toxin. Secondly, a DTR-UnaG-ODDD-HA construct (Oxygen-dependent degradation domain: ODDD), driven by the hypoxia dependent SXHRE promoter will be used. This system allows ablation of hypoxic cells through the DTR and identification of hypoxic cells through the UnaG fluorescent protein. Only cells that are hypoxic at the time of administration/analysis will be labelled. Both cell lines will be transduced with luciferase to allow identification of distant metastasis. Results and discussions The two systems for this project have been generated and transduced into the 4 T1 cell line. Lineage tracing and selective ablation have been shown to work in vitro and clones are being characterised. In vitro experiments have shown the HIF-1α-CRE-ER\textsuperscript{TAM} system to be extremely tightly regulated in an oxygen and tamoxifen dependant manner. Conclusion Once optimised, both systems will be used in an in vivo orthotropic mouse model to investigate the role of the hypoxic cells in breast cancer, metastasis and response to radiotherapy.

Abstracts

PO-274 DEVELOPMENT OF AN IN VITRO TUMOUR MODEL TO EVALUATE DRUG EFFICACY AND EPITHELIAL MESENCHYMAL TRANSITION IN TRIPLE NEGATIVE BREAST CANCER.

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Introduction The advent of diverse methods for 3-dimensional (3D) cell culture has allowed scientists to address some of the limitations of conventional 2D methods and have been applied to studies concerned with cancer therapy response. A major component of the tumour microenvironment overlooked during drug discovery and cancer studies is the interstitial fluid pressure (IFP). The aim of this project is to study breast cancer cell invasion and responsiveness to chemotherapeutic agents in a more physiologically relevant environment taking into account the effect of IFP and fluid flow.

Material and methods MDA-MB231 breast cancer cells were selected due to their triple negative subtype and were seeded in a dense (80 mg/mL) collagen scaffold, to form an ‘artificial cancer mass’ (ACM). The metabolic activity of the cells in 2D, 3D, static and under flow conditions: 100 and 500 μl/min: IFP: 19 mmHg were evaluated using an Alamar Blue assay. Markers of epithelial-mesenchymal transition (EMT) were determined using qRT-PCR and ΔCt values derived with reference to GAPDH. The responsiveness of the cells to docetaxel was assessed.

Results and discussions MDA-MB231 cells exhibited a decrease in metabolic activity when cultured in 3D compared to 2D. The 3D/flow environment promoted a switch from an epithelial to a mesenchymal phenotype as evidenced by increased expression levels of Snail (2 fold); vimentin (0.6 fold); MMP1 (0.7 fold); HIF-α (0.2 fold). In contrast, incorporation of IFP in the 3D/flow system was associated with decreased expression levels of vimentin (0.25 fold) and HIF-α (0.56 fold).