Fluorescent labelling of these cells allowed the tracking of their behaviour once placed into a 3D environment. Once recombined, these two cell types coalesced to form spherical and elongated duct-like structures. These structures comprised a layer of luminal cells surrounded by myoepithelial cells. Confocal imaging of myoepithelial and luminal markers revealed reconstitution of a bilayer structure absolutely reflective of normal physiology.

This novel 3D model of the human breast duct presents a powerful tool with which to dissect myoepithelial–luminal interactions in the early stages of breast cancer, and will help uncover markers to predict DCIS patients who will progress towards invasive breast cancer.

A comparative study of male versus female breast cancer identifies overexpression of eiF signalling pathways in male breast cancer providing opportunities for a therapeutic window

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Background Although rare, male breast cancer (MBC) is becoming more common, yet remains understudied. Treatments are informed by clinical studies conducted in women, based on assumptions that underlying biology is the same, although evidence suggests this may not be the case.

Methods A case-matched transcriptomic investigation of MBC and female breast cancer was performed. Transcriptomic data was confirmed by qRT-PCR and biomarkers assessed immunohistochemically on 477 MBC samples represented on tissue microarrays and related to survival. MicroRNA (miR) expression was determined in a subset of cases by RNA-seq.

Results Hierarchical clustering and Pathway Ingenuity Analysis identified gender-specific gene expression patterns. Expression of specific eIF transcripts was up-regulated in MBC, confirmed by qRT-PCR. By immunohistochemistry, eIF4E and eIF5 were negatively prognostic for overall survival ($p = 0.012$; HR = 1.77, 1.12–2.8 and $p = 0.033$; HR = 1.68, 1.04–2.74, respectively). Effects on overall survival remained independently prognostic upon multivariate analysis (eIF4E $p = 0.05$; HR 2.13 (1.0–4.6), eIF5 $p = 0.04$; HR 2.63 (1.04–6.65), respectively). Co-expression of eIF4E and -5 ($p = 0.005$; HR = 2.471, 1.280–4.770) also impacted on survival, remaining so upon multivariate analysis ($p = 0.002$; HR 6.205, 1.99–19.30). miR analyses on a subset of MBC cases revealed expression of 19 miRs which may regulate eIFs.

Conclusions Our findings support the notion that breast cancer is different between genders. Overexpression of eIF4E and eIF5 suggests these proteins could represent predictive biomarkers in MBC. With mTOR inhibitors which target this pathway now in the clinic, these biomarkers may represent new targets for therapeutic intervention, although further independent validation is required.

P-glycoprotein (ABCB1) expression during breast cancer development is regulated by HuR-dependent loading of miR-19b at non-canonical sites

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Chemotherapy resistance remains a major barrier to successful breast cancer (BC) treatment. Therapies that inhibit drug efflux pumps to overcome resistance have failed due to off-target side effects. Manipulating tumour-specific pathways of drug efflux pump expression could target chemoresistance, whilst simultaneously overcoming side effects. Our aim was to examine BC-specific, post-transcriptional regulation of P-glycoprotein (ABCB1), an efflux pump long associated with chemoresistance.

First, we determined the ABCB1 untranslated regions (UTRs) expressed in BC and established UTRs previously implicated as regulatory were broadly undetectable in primary BC samples or cell lines. Next, we screened for potential microRNA regulators by in silico prediction of binding to the expressed ABCB1 3′UTR. The candidate pool was refined using a BC cohort of 302 patients by eliminating microRNAs not expressed or without an inverse correlation to ABCB1 mRNA. We further screened by examining microRNA (qPCR) and P-glycoprotein (IHC) expression across a panel of matched normal breast, DCIS and invasive BC tissues. P-glycoprotein was significantly up-regulated across this progression, and one microRNA, miR-19b, was significantly both down-regulated and negatively correlated with P-glycoprotein, identifying it as the single candidate regulator.

Subsequent biochemical analyses found exogenous miR-19b overexpression reduced P-glycoprotein protein expression and enhanced intracellular epirubicin retention and associated cell death. Surprisingly, mutation of the predicted miR-19b seed region did not abrogate miR-19b-dependent regulation. In fact, sequential deletion of the UTR identified a miR-19b-responsive region devoid of a miR-19b seed site. *In silico* analysis revealed a consensus sequence for the RNA-binding factor HuR. Both activity and expression of HuR were essential for miR-19b loading to this non-canonical site.

Although miR-19b and HuR have been reported as putative oncogenes, their role in breast cancer appears more equivocal and targeting either could lead to chemoresistance.

Identifying molecular drivers and early diagnostic biomarkers in breast cancer leptomeningeal metastasis by the interrogation of cerebrospinal fluid

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Introduction Breast cancer leptomeningeal metastasis (BCLM) is particularly devastating, with a median survival of just 3–4 months. Timely diagnosis is often challenged by low sensitivity of cerebrospinal fluid (CSF) cytology. Further, translational progress is hampered by the lack of in vivo BCLM models.

Methods CSF ($\leq$ 10 mL) and plasma were collected from patients with confirmed BCLM. Following protocol optimisation, cell-free DNA (cfDNA) was extracted using Qiagen Circulating Nucleic Acid Kit, quantified by Qubit fluorometer and fragment length assessed by the Agilent Bioanalyzer. Primary human breast cancer cells were injected intravenously into NSG mice. Mouse skull caps were formalin-fixed before dissecting out the meninges, which were stained for human CK19 and lamin A/C, and whole-mounted for confocal immunofluorescent imaging.