Project Details	
Project Code	PHS27Br Lloyd-Lewis
Title	Investigating mechanisms of cell competition in breast cancer susceptibility and early tumour initiation
Research Theme	Population Health Sciences
Project Type	Wet lab
Summary	How do cancers grow into space occupied by normal cells? Competition between tumour and non-tumour cells plays an important role, allowing tumour cells to eliminate normal cells and grow in their stead. Yet the underlying molecular mechanisms remain unclear. In this interdisciplinary project, the student will investigate mechanisms controlling cell competition, breast tumour initiation and progression through combining advanced methods in genetic epidemiology with lab-based cell biology techniques using cancer cell lines, pluripotent stem cells, patient-derived 3D primary organoids and in vivo models.
Description	IMPORTANCE Interactions between cancer cells and their non-cancerous counterparts play pivotal roles in modulating tumour growth. We now know that cancer cells can compete with and kill neighbouring host cells, and that elimination of 'normal' host cells is important to make space for tumour growth. This suggests that strategies that inhibit tumour-host cell competition, or that enhances the ability of non-cancer cells to outcompete adjacent cancer cells, might provide new ways of blocking tumour expansion. A better understanding of the mechanisms underpinning bidirectional communication between tumour and non-tumour cells therefore has the potential to identify novel and complementary therapeutic strategies. The tumour suppressor TP53 has been shown to turn cells into 'winners' that eliminate their neighbours in both in vivo and in vitro contexts. We recently performed a genome-wide CRISPR knockout screen in wild-type pluripotent stem cells undergoing competition with TP53 knock out cells and identified ~184 genes that upon mutation inhibit wild-type cell elimination, including several candidates implicated in cancer. Example candidates include genes involved in breast cancer relevant signalling pathways and BRCA1/2-mediated DNA repair. This project aims to validate the competitive interactions of the identified genes and investigate their mechanisms of action in powerful breast cellular models, with a focus on genes implicated in BRCA1/2 breast cancer susceptibility and tumour initiation. Because individuals with germline BRCA1 or BRCA2 mutations face a high risk of breast cancer, often prompting consideration of drastic preventive measures such as prophylactic mastectomy, identifying the mechanisms underlying these genes' anti-tumour effects could reveal new avenues for breast cancer prevention in high-risk individuals. Additionally, this will provide a deeper understanding of the role of cell competition in breast cancer development and provide researchers with new drug targets for TP53 and BRCA1/2 tumours. RESEARCH TRAINING This PhD project takes a cross-disciplinary approach, combining population-level genetic epidemiology, bioinformatics and laboratory-based techniques in molecular and cell biology. The student will

	therefore acquire a versatile and highly sought-after skill set during their PhD. Aim 1. Use genetic epidemiological approaches to determine whether candidate cell competition genes are causally associated with breast cancer Training in genetic epidemiology will be undertaken at the world leading MRC Integrative Epidemiology Unit at the University of Bristol, supervised by co-supervisor Dr Richmond. Mendelian Randomization (MR) will be performed to interrogate whether candidate cell competition genes and pathways already identified are causally associated with breast cancer. Alongside using the largest genetic dataset of breast cancer available (BCAC), they will use the CIMBA dataset focused on genetic modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers. This powerful dataset ensures sufficient sample sizes for reliable analyses, with data available for approx. 90,000 individuals (with over 58,000 genotyped to date). Importantly, CIMBA has extended its research into the effects of other cancer susceptibility genes relevant to this project, including TP53, ATM and PALB2. The Cancer Genome Atlas (TCGA) will also be used to compare expression in breast tumours and normal breast tissues for genes identified as cell competition modulators and with evidence of a causal effect of expression on breast cancer risk. Aim 2. Characterise the competitive function and mechanisms of candidate genes in breast tissue relevant cell-based models Training in laboratory methods will enable the student to investigate the molecular mechanisms underpinning the competitive function of prioritised genes identified as being causally associated with breast cancer (Aim 1). Using cellular models, including normal, mutant BRCA and mutant TP53 cell lines, the student will assess the consequences of reducing the expression of prioritised genes on cell competition phenotypes by measuring cell survival, apoptosis, quantitative cell dynamics, and transcriptomic changes in knockdown cells using RNA sequencing. In addition, the student will establish a three-dimensional (3D) organoid model of cell competition. This will initially be derived through differentiation of human pluripotent stem cells, and from primary mammary cells isolated from wild-type and BRCA1 mutant mouse models (available through collaborators at ICR), before being extended to patient-derived 3D breast organoids. Through this work, the student will gain expertise in a broad range of experimental techniques, including 2D/3D cell culture, fluorescence confocal microscopy, flow cytometry, immunohistochemistry, RNA sequencing, image analysis, and bioinformatic analysis of RNA-sequencing datasets. Aim 3. Determine whether identified cell competition mechanisms are relevant and causally associated with other epithelial cancer types Following training received in earlier aims, the student will steer their project to prioritise tractable genes and signalling networks of interest to them for further mechanistic investigation using techniques established in Aim 2, alongside new methods as required. The resulting data and findings will also inform further genetic epidemiological analyses. For example, whether differentially expressed genes and pathways

	identified by RNA-sequencing experiments in Aim 2 are causally associated with breast and other cancer types can be investigated using MR and validated in cancer patient datasets (e.g. TCGA).
Can be completed part time?	Yes
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