Unravelling the role of cancer associated fibroblasts in cancer evolution and drug resistance using microfluidics

Supervisors:

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Research Summary

Cancer associated fibroblasts (CAFs) secrete proteins that promote drug resistance and the evolution of cancer cells. In some cancers such as pancreatic ductal adenocarcinoma (PDAC), stromal cells greatly outnumber cancer cells within tumours. Current attempts to study clonal evolution and heterogeneity involves growing large populations of cells that are individually genetically barcoded and quantifying barcodes following selection pressure as a measure of heterogeneity. While the Banerji Lab uses these experimental models, such methods do not lend themselves to studies with co- cultured cell types e.g., cancer cells and CAFs. Technologies that help us study how CAFs drive evolution and heterogeneity in PDAC is an unmet need.

Commonly used co-culture systems such as mixed cultures in flasks or Transwell membrane systems have a number of limitations that make them poorly suited to evolution and drug resistance studies including: a) variability in the degree of paracrine signalling & physical contact between cell types since growth is often patchy and in the case of membrane systems, independent populations lack physical co-contacts altogether, b) difficulty exploring dynamics since tracking the state of individual cells and their progenitors in macroscale over time is challenging, and c) are not scalable to high throughput screening. Droplet based microfluidics (Fig. 1) can address all of the above issues. This technique involves the controlled formation of nanolitre-femtolitre (10^{-9}-10^{-15} L) droplets segregated by immiscible oil. Droplet microfluidics is capable of precise control over co-culture conditions (i.e. 1:1 cancer cell:CAF within in each droplet), allows for the easy tracking of individual cells since each droplet acts as an isolated bioreactor, and is scalable, capable of generating >20,000 droplets per second. No droplet microfluidic platforms however have been developed to study cancer evolution and drug resistance within CAF and tumour co-cultures.

We have also previously used pooled whole genome CRISPR screens to find genes relevant to drug resistance. However, this technology has not been applied to finding genes within cancer cells that are key to drug resistance induced by CAF co-cultures. Following droplet microfluidic screens, we will establish a new methodology where following transfection of a pool of lentiviruses, cell cultures of pancreatic cancer cells will be cultured in a perfusion system to allow identification of genes within cancer cells that cause sensitivity or resistance to anticancer drugs upon contact with secreted proteins from CAFs.

Hypothesis: Co-culture of cancer cells and CAFs using novel platforms will enable the study of how cancer associated fibroblasts (CAFs) affect PDAC evolution and drug resistance.

Aim 1. To develop a droplet microfluidic device for generating, merging and arraying single cell droplets of PDAC and CAFs

You will develop a novel microfluidic system (Fig. 1) to: a) generate nanolitre droplets containing on average 1 cell (examples of PDAC cell lines to be used include CAPAN-1 & AsPC-1), or pancreatic stellate cell (CAF) per droplet in an aqueous/oil/aqueous or aqueous/oil format using T-junction microchannels, b) merge two droplets using a passive wall step trap that releases merged droplets due to changes in local pressure, c) array up to 1M merged droplets on a planar microreservoir where they can be cultured and imaged using fluorescent microscopy. Aqueous droplets will be generated using a gelatin methacrylate hydrogel that supports cell adhesion and can be gelled at will using low incident energy UV light. This system will be used for aims 2 and 3. A macroscale perfusion bioreactor co-culture system will be generated in later years to support CRISPR screen experiments (Fig.1, right) to be conducted in Aim 4.

Aim 2. To establish the role of pancreatic CAFs in establishing intratumoural heterogeneity caused by commonly used anticancer drugs in PDAC

You will GFP-label pancreatic cancer cells lines and co-culture these individually or with pancreatic CAFs in merged droplets. A concentration of PDAC standard of care drugs (Gemcitabine, oxaliplatin, irinotecan, 5-FU and paclitaxel) required to reach 80% cell death in a population of GFP-labelled PDAC cells (read by quantifying GFP using fluorescent microscopy) will first be determined in tumour monoculture droplets. We will then grow GFP labelled pancreatic cancer cells (starting with single cells/droplet) in aqueous droplets and fuse these with droplets with non GFP-labelled pancreatic cancer cells (control) or pancreatic CAFs. The fused droplets will be exposed to drugs as above. The student will then quantify the number of cancer cells in each droplet using automated fluorescent microscopy which will provide a measure of heterogeneity of resultant populations derived from single GFP labelled cancer cells grown with, out without CAFs.

Aim 3. To study the role of pancreatic CAFs in causing drug resistance in pancreatic cancer cells.

GFP-labelled PDAC cells will be grown in aqueous droplets and fused with droplets with non GFP labelled pancreatic cancer cells (control) or droplets with CAFs. A panel of 100 anti-cancer drugs will be screened to determine GI50s and hits will be considered if the GI50 is more than two-fold different between the pancreatic cancer cells grown in non- GPF labelled pancreatic cancer cells in co-culture or CAF co-cultures.

Aim 4. CRISPR screen to identify genes in pancreatic cancer cells responsible for resistance/sensitivity to anticancer drugs when pancreatic cancer cells are exposed to pancreatic CAFS

A culture of stably CAS9 transfected PDAC cells will be generated. Cells will then be transfected with a lentiviral genome wide pooled CRISPR library and exposed to 1 or 2 standard of care drugs or those that demonstrated differential sensitivity when exposed to cancers or CAFs in aim 3. The perfusion system developed above expose transduced cells in the CRISPR library to media conditioned by other pancreatic cancer cells (control) or pancreatic CAFs. Because of the longer duration of culture and suitability for pooled instead of single cell analysis, the macroscale perfusion system is better suited to this aim than the droplet microfluidic system developed for

earlier aims. Following 2-3 weeks of culture, the pancreatic cancer exposed to the CRISPR pool will be analysed using targeted next generation sequencing identify genes associated with resistance to the anticancer drug being studied post-exposure to condition media. Any hits of genes will be validated by single CRISPR knockouts.

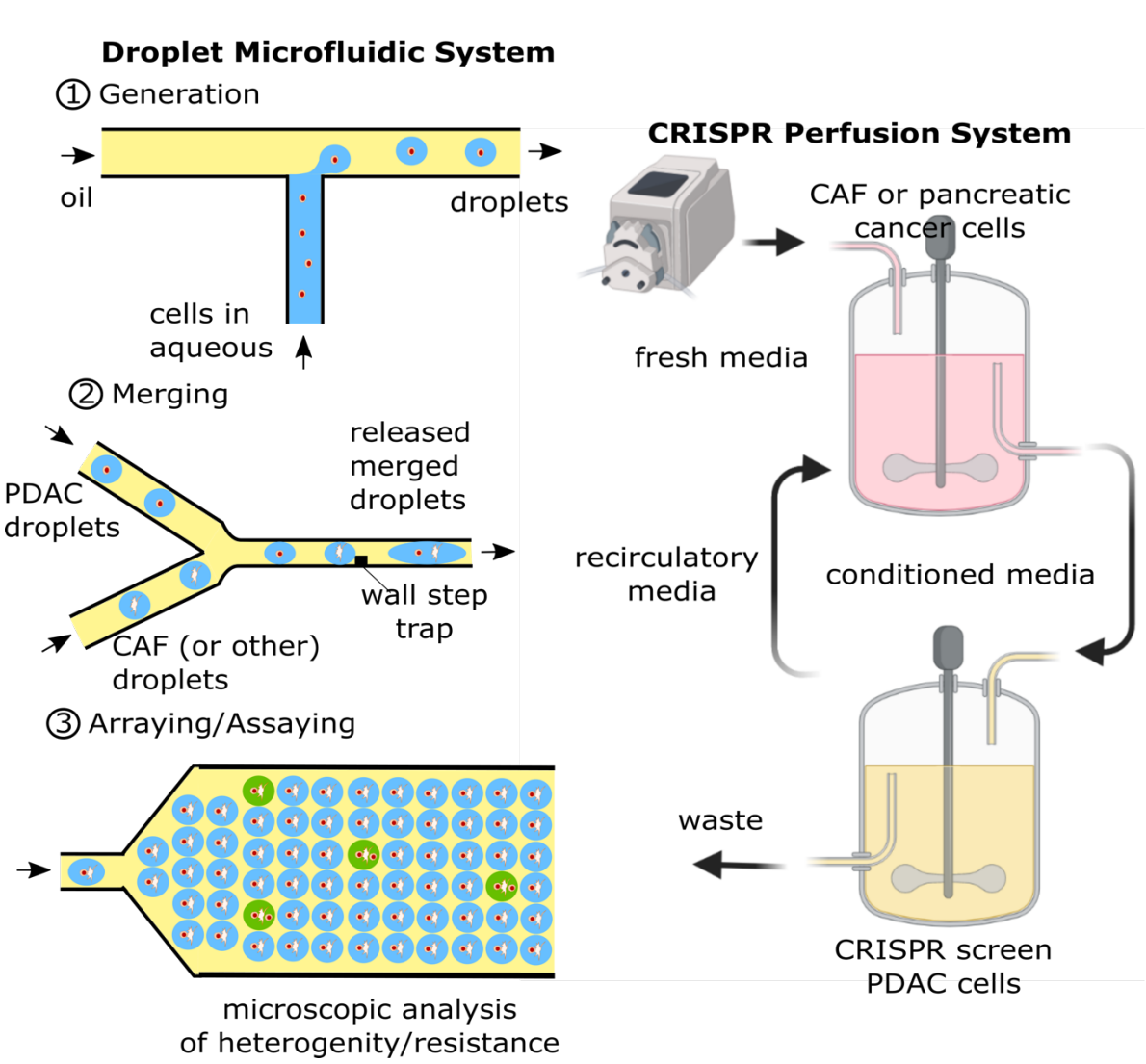

Figure 1: Droplet microfluidic platform (left) for 1) generating nL droplets containing mostly 1 cell droplets, 2) droplet merging using a passive wall step occlusion that forces droplets to coalesce and 3) arraying for incubation and microscopy analysis. CRISPR perfusion platform (right) capable of continuous feed of CAF conditioned media to pancreatic cancer cells.

Literature references

1. *Shang et al. Emerging Droplet Microfluidics. Chem. Rev. 2017, 117, 7964−8040. DOI: 10.1021/acs.chemrev.6b00848*
2. *Acar A et al. Exploiting evolutionary steering to induce collateral drug sensitivity in cancer. Nature communication 2020, 11:1923. DOI: 10.1038/s41467-020-15596-z*

Person specification

This project is suitable for a talented graduate or undergraduate student with life sciences, engineering, or physics background. The standard minimum entry requirement is a relevant undergraduate honours degree (First or 2:1). Applications are invited from talented graduates or final year undergraduates. We particularly welcome British applicants from Black and ethnic minority backgrounds, as they are underrepresented at PhD level within Imperial and The Institute of Cancer Research.

The studentship will be registered at the Institute of Cancer Research with affiliate status at Imperial College London. The student will have access to both institutions and benefit from the world class research infrastructure and expertise across the two institutions. The student will become a member of the CRUK Convergence Science Centre PhD cohort which is a unique group of students working across distinct disciplines to tackle the big problems in cancer. A unique convergence science training programme will provide the skills and language to navigate different disciplines.

Funding and Duration

Studentships will be for four years commencing in October 2022. Successful candidates will undertake a four-year research training programme under the guidance of a supervisory team of world-class researchers. Students will receive an annual stipend, currently £21,000 per annum, and project costs paid for the four-year duration. Convergence Science PhDs cover tuition fees for UK students only. Funding for overseas fees is not provided, international students are invited to apply subject to outlining how they will meet the difference in tuition fees.