TUMOUR MICROENVIRONMENT AND PROTEOMICS

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High grade serous ovarian cancer (HGSOC) and triple negative breast cancer (TNBC) have limited treatment options, as only few targeted therapies effectively kill cancerous cells and patients frequently develop resistance to standard therapies. The tumour microenvironment actively supports cancer pathology and is populated by a variety of cell types that also offer alternative routes for therapy. Our research focuses on cancer-associated fibroblasts (CAFs), as we and others have shown that they play a major role in modulating cancer pathology. CAFs strongly influence the function of cancer and other stromal cells by secreting extracellular matrix (ECM) components and modifiers, soluble factors and extracellular vesicles (EVs). We aim to understand the molecular mechanisms through which CAFs support cancer, and envisage targeting CAFs in combination with other anti-cancer therapies as a promising strategy to stop cancer growth and metastasis.

Our research primarily focuses on the role of CAFs in HGSOC and TNBC. These tumours contain vast regions of stroma, which are densely populated by CAFs, while CAFs were shown to play active roles in the progression of both diseases. Importantly, HGSOC cells and TNBC cells have few recurrent mutations, therefore limiting the availability of targeted therapies against cancer cells. As such, CAFs offer a valid alternative therapeutic opportunity in these tumour types (Santi *et al*., 2018, *Proteomics*; Domen *et al*., 2021, *Cancers*). We aim to decipher how CAFs create a tumour-promoting microenvironment and how we can block this process to make the tumour microenvironment unfavourable to cancer growth and tumours more vulnerable to therapeutic treatments; our overarching goal is to determine strategies that target CAFs to stop cancer.

CAFs can originate from normal fibroblasts resident at the site where the primary tumour develops. When a tumour starts developing, normal fibroblasts become activated into CAFs, and become able to secrete a plethora of soluble factors and ECM components that influence the function of surrounding cells and actively support cancer progression (Figure 1) (Kugeratski *et al*., 2022, *Science Signaling*; Santi et al., 2018, Proteomics). While CAFs are the results of the reprogramming of normal cells, we aim to find ways to revert CAFs to a normal cell-like phenotype that does not support cancer and that improves response to therapies.

For our research, we mostly use CAFs that we isolate from tumour tissues that are kindly donated by patients for research purposes, and we develop clinically relevant models to study their functions (Neilson, Cartwright *et al*, 2023, *Matr Biol Plus*). Our group has a strong expertise in mass spectrometry (MS)-based proteomics (van den Biggelaar *et al*., 2014, *Blood*; Patella *et al*., 2015, *Mol Cell Proteomics*; Diaz *et al*., 2017, *J Cell Sci*; van der Reest, Lilla *et al*., 2018, *Nat Commun*), and we integrate this innovative technology in our research to tackle the above questions and provide new levels of understanding of CAF biology.

CAF – tumour blood vessel interaction

The vasculature of solid tumours is often responsible for the progression and aggressiveness of disease. Initially, tumours recruit blood vessels to obtain nutrients and oxygen to sustain proliferation. Later on, the tumour vasculature becomes leaky and provides a route for cancer cells to escape and form distant metastases.

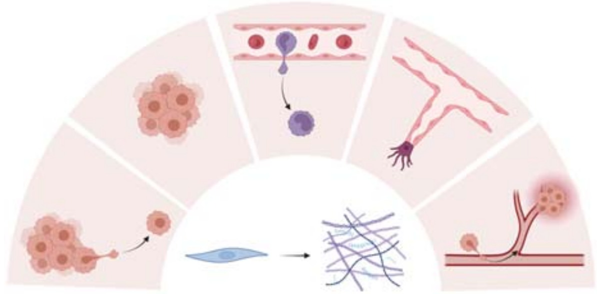

Figure 1. CAFs influence the behaviour of cancer and endothelial cells. Cartoon showing that cancer associated fibroblasts (CAFs), particularly those that secrete abundant ECM, influence various aspect of tumour development. Our works have shown that CAFs regulate mechanisms such as (from left to right): growth and invasive behaviour of the cancer cells, recruitment of immune cells into the tumour from the circulation, sprouting angiogenesis, cancer cell intravasation from the primary tumour to form distant metastasis.

Endothelial cells (ECs) line the inner layer of the vessel wall and regulate the functionality and growth of the vessel. Tumour blood vessels are typically embedded within a CAF-rich stroma, such that ECs are exposed to factors that CAFs secrete. We have found that CAFs influenced EC function by transferring functional proteins through a specific subset of EVs that are bound to the ECM that they produce. In particular, CAFs could transfer membrane-bound proteins that confers the ability to the endothelium to interact with monocytes, which influence aspects of tumour progression, including antitumor immunity and metastasis. We therefore discovered a novel mechanism that could be targeted in CAFs to oppose the formation of tumour-promoting microenvironment (Santi, Kay *et al*., 2024, *Sci Signal*).

CAFs & metabolism

Altered metabolism is a hallmark of cancer. In the last few years, it has emerged that, in addition to the metabolism of cancer cells, the metabolism of stromal cells is also an important regulator of cancer pathology (Kay *et al*., 2023, *Curr Opin Biotechnol*; Kay *et al*., 2021, *Front Oncol*; Kay & Zanivan, 2021, *Curr Opin Syst Biol*). Epigenetic regulators, such as histone acetylation and methylation, play major roles in determining cell phenotypes and functions, including in CAFs. An interesting aspect of cell metabolism is its link to epigenetics, as it provides acetyl and methyl groups as substrates for histone modifications. We found that CAFs produced high levels of acetyl-CoA, a source of acetyl groups for protein acetylation, and that this triggered the activation of a transcriptional programme resulting in the production of tumour-promoting ECM (Kay *et al*., 2022, *Nature Metabolism*). We are now further investigating the potential of targeting mechanisms activated downstream of acetyl-CoA production in CAFs to block tumour development.

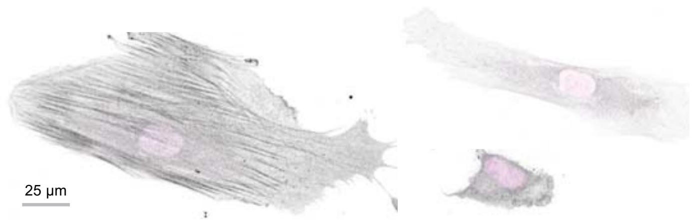

Figure 2. CAFs have different phenotypes. αSMA staining (marker of myCAF) of patient-derived HGSOC CAFs in culture suggesting that the bigger cell is a CAF with a myCAF phenotype while the elongated one is a CAF with an iCAF phenotype. Scale bar = 50 µm.

OmGel: a clinically relevant tool to study CAF biology

In patient samples, CAFs have different phenotypes and functions (Figure 2), some of which are interchangeable. A key example are myCAFs, which produce abundant ECM (Figure 1), and iCAFs, which have an inflammatory gene expression signature. Not much is known about iCAF functions in cancer because of the difficulty to maintain their phenotype in normal culture conditions. In collaboration with the Salo team at the University of Helsinki, we have developed omentum gel (OmGel), a clinically and physiologically relevant ECM made from the omentum (a major organ where HGSOC cells metastasise and that is removed during debulking surgery) of patients with HGSOC (Neilson, Cartwright *et al*., 2023, *Matr Biol Plus*). We showed that OmGel has unprecedented similarity to the ECM of HGSOC tumours and that it supports HGSOC cells' invasive behaviour. Importantly, CAFs cultured with OmGel maintained an iCAF phenotype. Therefore, OmGel will uniquely enable us to study iCAF functions to advance our knowledge on their role in cancer.

News

This year, Paula, Kunal and Ashton have joined our team to push forward our research on cancer metabolism. Moreover, Emily Kay has presented her work on the immune-regulatory roles of CAFs in breast cancer at the International Beatson Cancer Conference in Glasgow and ISCaM2023 - Systemic Metabolism and Cancer Meeting in London.

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