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 other 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, ECM 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 cancer cells as a promising strategy to hamper 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 pro-tumorigenic 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 for therapy.

CAFs can originate from normal fibroblasts resident at the site where the primary tumour develops. When a tumour starts developing, normal fibroblasts become activated. This activation induces extensive reprogramming of gene expression and protein levels, such that CAFs become able to secrete a plethora of soluble factors and ECM components (Figure 1) that actively support cancer progression. CAFs were also shown to secrete EVs whose cargos could aid tumour progression by supporting cancer cell growth and invasion (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.

To understand how to target CAFs in tumours, it is essential that we understand how CAFs make the tumour microenvironment pro-tumorigenic and pro-metastatic, and what the molecular mechanisms are that sustain CAF functions. Our major interest is the role of cell metabolism (Kay *et al.*, 2021, *Front Oncol*; Kay & Zanivan, 2021, *Curr Opin Syst Biol*) and extracellular vesicles secreted by CAFs. For our research model, we mostly use CAFs that we isolate from tumour tissues that were kindly donated by patients for research purposes (Hernandez-Fernaud, Ruengeler *et al.*, 2017, *Nat Commun*; Kugeratski *et al.*, 2019, *Science Signaling*). 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*; Hernandez-Fernaud, Ruengeler *et al.*, 2017 *Nat Commun*; 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.

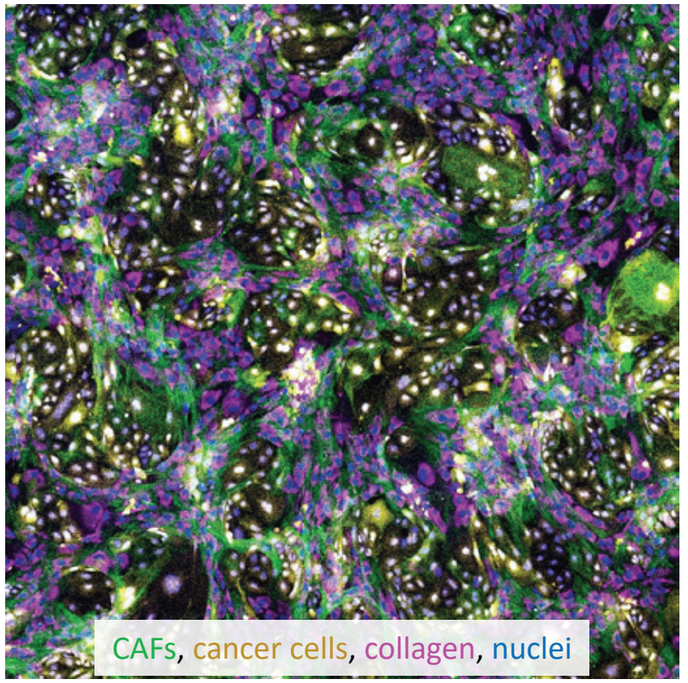

Figure 1
CAFs-cancer cells co-culture
Immunofluorescence image of high grade serous ovarian cancer (HGSOC) cancer cells and patient-derived CAFs, showing that CAFs produce high amounts of collagen. Purple = collagen, Yellow = HGSOC cells, Green = CAFs, Blue = Nuclei.

Image by Teresa Glauner.

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.

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 directly interact with CAFs or are exposed to the factors that they secrete. Our group previously showed that CAFs secreted proteins that influence blood vessel growth and functionality via altering endothelial cell behaviour (Hernandez-Fernaud, Ruengeler *et al.*, 2017, *Nat Commun*; Kugeratski *et al.*, 2019, *Sci Signal*). We have also shown that the ECM secreted by CAFs play an active role in the metastatic dissemination through facilitating the binding of the cancer cells to the blood vessels (Reid *et al.*, 2017 *EMBO J*). We have now found that CAFs also influenced EC function by transferring functional proteins through EVs. In particular, CAFs can transfer plasma membrane and membrane-bound proteins to the surface of the endothelial cells. This process confers the ability to the endothelium to interact with other cell types, such as monocytes, which influence aspects of tumour progression, including antitumor immunity and metastasis. We therefore discovered another way through which CAFs make ECM pro-tumorigenic and we are investigating this aspect further.

CAFs & metabolism

Metabolic alterations are a well-established hallmark of cancer. In the last few years, it has emerged that, in addition to the metabolism of cancer cells, also the metabolism of stromal cells is an important regulator of cancer pathology (Kay *et al.*, 2021, *Front Oncol*; Kay & Zanivan, 2021, *Curr Opin Syst Biol*). Epigenetic regulators, such as histone acetylations and methylations, 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 metabolites, such as 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 pro-metastatic ECM (Kay *et al.*, 2020, *bioRxiv*). We are now further investigating the potential of targeting acetyl-CoA production in CAFs in cancer.

News

This year, Teresa Glauner presented her work on linking collagen-producing cancer-associated fibroblasts and immune cells at the British Society for Immunology Congress 2021. Moreover, we have been awarded a CRUK Early Detection and Diagnosis Project Grant to work in collaboration with the Proteomics team, the Liver Disease and Regeneration team (Tom Bird), the Computational Biology team (Crispin Miller) as well as University of Oxford and University of Nottingham to identify biomarkers for early detection of liver cancer.

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