TUMOUR MICROENVIRONMENT AND PROTEOMICS

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In solid tumours, cancer cells are embedded within a stroma populated by different cell types. Cancer associated fibroblasts (CAFs) are a major non-neoplastic stromal cell population, which our lab and other groups have shown play crucial roles in cancer progression. In fact, CAFs have a unique ability to establish crosstalk signalling with cancer cells and other stromal cells by secreting soluble factors, extracellular matrix (ECM) components and modifiers, and physically interacting with surrounding cells. Thus, our research focuses on CAFs; we envisage that targeting CAFs rather than, or in combination with, cancer cells is a promising innovative strategy to hamper cancer growth and metastasis.

Our research primarily focuses on the role of CAFs in breast and high-grade serous ovarian cancers because these tumours contain a sizeable proportion of stroma, which is densely populated by CAFs. Furthermore, CAFs have been shown to play key functional roles in the progression of both diseases. Importantly, ovarian cancer cells lack recurrent somatic mutations and this limits the availability of targeted therapies against the cancer cells. Therefore, CAFs may offer a valid alternative therapeutic opportunity in this tumour type.

We aim to decipher how CAFs contribute to tumour progression and metastasis, with the ultimate goal of identifying strategies to target these cells for therapy.

In particular, we study how CAFs promote invasive behaviour of the cancer cells and support their uncontrolled proliferation and survival, and how CAFs influence endothelial cell (EC) behaviour. ECs are a key cellular component of the blood vessels. ECs line the inner layer of the vessel wall and regulate the functionality and growth of the vessel. In many solid tumours, the vasculature is responsible for the progression of the disease. Initially, tumours recruit blood vessels to obtain nutrients and oxygen to sustain the uncontrolled growth of the cancer cells. Later on, the tumour vasculature becomes leaky and provides a route for the cancer cells to escape and form distant metastases. We also study the role of cell metabolism in the regulation of CAF function.

Our group has a strong expertise in mass spectrometry (MS)-based proteomics, and we integrate this innovative technology in our research to provide new levels of understanding of CAF biology.

CAFs can originate from the normal fibroblasts resident at the site where the primary tumour develops. Under stress conditions, such as chronic stimulation by factors secreted by the cancer cells, redox stress and hypoxia, the normal fibroblasts become activated. This activation induces extensive reprogramming of gene expression and protein levels, such that CAFs are characterised by a) being highly contractile, and b) secreting soluble factors and ECM components that promote the progression of cancer. This highlights the importance of a better understanding of how CAFs alter the tumour microenvironment and how the surrounding stromal and cancer cells react to these changes. To tackle this question, we make extensive use of state-of-the-art MS–proteomics approaches, which we have previously shown to be a powerful technology to investigate cellular secretomes (Zanivan *et al.,* Mol Cell Proteomics 2013; 12: 3599–611) and molecular mechanisms underpinning EC functions (van den Biggelaar *et al.*, Blood 2014; 123: e22–e36 Patella *et al.,* Mol Cell Proteomics 2015; 14: 621–34; Patella *et al.,* J Proteome Res 2016; 15: 2187–97).

Pro-invasive functions of CAFs

Using methods that we have previously developed for in-depth quantitative MS–proteomic analysis of secreted proteins in cell culture (van den Biggelaar *et al.,* Blood 2014; 123: e22–e36) we have provided the first global portrait of proteins that are differentially secreted when patient-derived mammary fibroblasts are activated into CAFs by breast cancer cells. We have found that the chloride intracellular channel protein 3 (CLIC3) is one of the most upregulated and heavily secreted proteins in CAFs. Moreover, we have established an unprecedented role for this protein in the extracellular environment: CLIC3 is a pro-invasive oxidoreductase able to promote tumour invasion and vessel growth by increasing ECM stiffness though the reduction (activation) of the extracellular transglutaminase TGM2. Importantly, we have found that CLIC3 is highly abundant in the stroma of triple-negative breast cancer and high-grade serous ovarian cancer (HGSOC) patients. Indicating that CLIC3 may have a role in the progression of the disease, high levels of CLIC3 in the stroma correlate with poorer patient prognosis (see Fig. 1). We are currently investigating the role of stromal CLIC3 in HGSOC to understand whether it is a potential novel target for this type of cancer, particularly for blocking metastasis, which is the major cause of death for this tumour type.

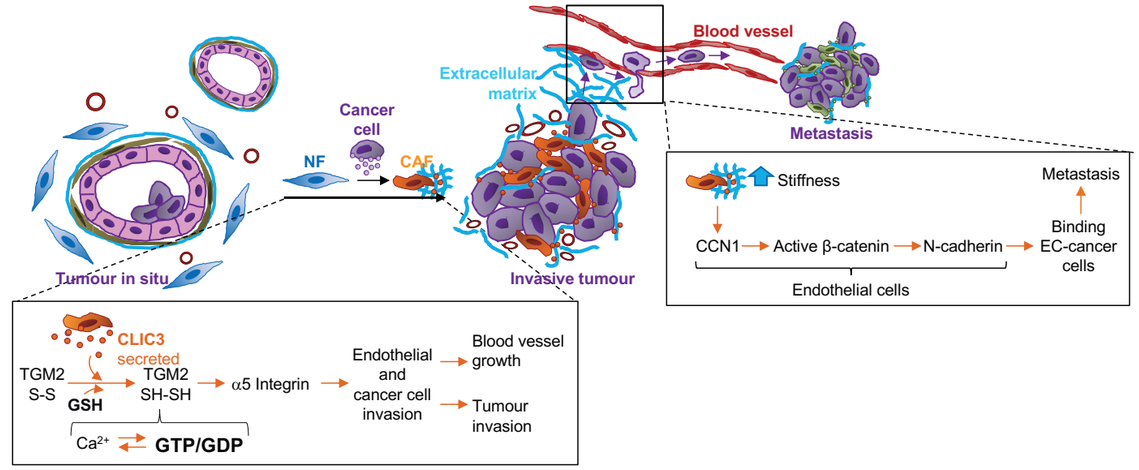

Figure 1
This cartoon summarises our recent findings in the context of tumour progression (via CLIC3, on the left) and metastasis (via CCN1, on the right). NF = normal fibroblasts; CAF = cancer associated fibroblasts (Adapted from Reid *et al.,* EMBO J 2017; 36: 2373–89)

Recently, we started investigating how hypoxia, which is typical in aggressive cancers, influences CAF functions. Using 3D co-cultures of CAFs and ECs, we found that hypoxia exacerbates the pro-angiogenic function of CAFs. This is important because excessive tumour angiogenesis can cause the formation of leaky blood vessels, which can worsen hypoxia and facilitate cancer cell intravasation into the blood flow to form distant metastasis. Extensive proteomic analysis of hypoxic breast cancer CAFs has pinpointed possible mechanisms underpinning this hypoxia-induced function, which we are currently investigating further.

Tumour stiffness favours cancer cell invasion

The CLIC3/TGM2 pathway that we have discovered promotes endothelial and cancer cell invasion by stiffening the tumour ECM. These results support previous findings that high stiffness promotes tumour invasion and metastasis. While several works have described how stiffness promotes invasive behaviour of cancer cells, it is largely unknown how stiffness controls EC behaviour and whether this impacts on tumour metastasis. We have discovered that high ECM stiffness increases levels of heterotypic cell–cell adhesion receptors on the surface of the ECs. In particular, we have characterised an unprecedented mechanism through which high stiffness increases expression and exposure to the plasma membrane of N-cadherin via upregulation of CCN1. Importantly, we showed that the CCN1/N-cadherin pathway facilitates the binding of the cancer cells to blood vessels, which is the first step of cancer cell intravasation into the blood stream for the formation of distant metastasis (see Fig. 1). We have therefore identified a new function of tumour stiffness on the vasculature and discovered a pathway that can be targeted to reduce metastasis by blocking stiffness-induced intravasation of cancer cells.

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