The tumour stroma is composed of many different cell types and extracellular matrix (ECM) components that work in concert to generate a microenvironment permissive to tumour initiation, progression and metastasis. Cancer associated fibroblasts (CAFs), the most abundant non-neoplastic cells in tumours, are characterised by their ability to secrete a plethora of factors that contribute to the formation of an aberrant tumour microenvironment and alter the behaviour of the surrounding cells. For this reason, CAFs play a key role in cancer pathology. Our group aims to identify molecular mechanisms that control the ability of CAFs to create a pro-invasive tumour microenvironment and to investigate the possibility of targeting these cells for therapy.

In particular, we study: 1) factors secreted by CAFs that can modify the tumour microenvironment and affect the behaviour of and communicate with the surrounding cells; and 2) how CAFs influence endothelial cell (EC) behaviour. ECs line the inner layer of the vessel wall and play a crucial role in regulating the function and growth of the vessel. In many solid tumours, the vasculature is responsible for disease progression. Initially, tumours recruit blood vessels to obtain nutrients and oxygen to sustain cancer cell growth. Later, the tumour vasculature becomes leaky and provides a route for the cancer cells to escape and form distant metastases.

Our group exploits its experience in high resolution mass spectrometry (MS)-based proteomics and accurate quantification methods, including stable isotope labelling with amino acids in cell culture (SILAC), in combination with in vitro and in vivo approaches to shed new light on the complexity of the tumour microenvironment.

CAFs can originate from normal fibroblasts resident at the site where the primary tumour develops. Under stress conditions, such as chronic stimulation by factors secreted by cancer cells, normal fibroblasts become activated. This activation induces extensive reprogramming of gene expression and protein levels such that CAFs are characterised by being highly contractile with many secreting soluble factors and ECM components that promote cancer progression. This highlights the importance of a better understanding of how CAFs alter the tumour microenvironment. To do this, we have used unbiased MS-proteomic approaches, which we have previously shown to be a powerful tool to investigate cellular secretomes and molecular mechanisms regulating EC functions, together with functional in vitro and in vivo studies that we have discovered and characterised previously unknown mechanisms through which CAFs drive invasion.

Unravelling CAF-induced paracrine mechanisms of cell invasion

We have developed methods to perform in-depth quantitative proteomic analysis of secreted proteins and ECM components, and used these to identify proteins abnormally secreted by fibroblasts when activated by cancer cells. Since CAFs are highly abundant in the stroma of breast cancers, we have investigated the process in this tumour type. We have identified more than 10,000 proteins secreted by fibroblasts, of which around 300 had altered levels in CAFs. These included ECM components and growth factors, such as collagens, fibronectin and transforming growth factor β, as well as well-known CAF markers. Intriguingly, we identified that the chloride intracellular channel protein 3 (CLIC3) was secreted by CAFs and deposited in the ECM. We have now shown that extracellular CLIC3 is a pro-invasive redox enzyme able to promote vessel growth and tumour invasion by activating the extracellular transglutaminase, TGM2 (Fig. 1) (in collaboration with Jim Norman’s group).

Detailed analysis of CLIC3 expression in different cancer types revealed that it is highly expressed in the stroma of aggressive breast and ovarian cancers, and that high levels of CLIC3 in these tumours predict poor patient outcome. We are now investigating the function of CLIC3 in the progression of tumours and the possibility of targeting it to block the pro-invasive function of CAFs in tumours.

Tumour stiffness favours tumour cell invasion

CAFs secrete a number of ECM components and ECM modifiers, such as cross-linking enzymes, which are responsible for the increased stiffness of tumours, a phenomenon that has been observed in many cancer types during progression. Blood vessels play a key role in the formation of metastasis, because cancer cells must intravasate into the blood stream to spread to distant sites (Fig. 2). It has previously been shown that high matrix stiffness enhances tumour invasion by promoting the invasive behaviour of cancer cells. However, it is not known if/when high stiffness can directly affect endothelial cell function related to tumour invasion.

Using MS proteomics, we have measured proteomic changes occurring in ECs when adhering to fibronectin-coated polycrylamide gels of physiological and tumour stiffness. Similar to studies with other cell types, our analysis showed that high stiffness induces proliferation and cell-ECM adhesion. Moreover, it revealed that several receptors involved in cell-cell interactions were upregulated by tumour stiffness. We have characterised one mechanism through which high stiffness induces increased levels of a member of the CCN protein family that in turn enhances the expression and exposure to the plasma membrane of a transmembrane receptor of the cadherin family. We show that this mechanism is key for the binding of cancer cells to ECs, which is the first step in cancer cell intravasation into the blood stream, and favours the formation of metastasis. Hence, we have identified a new function of tumour stiffness on the vasculature and discovered a pathway that can be targeted to reduce and possibly block stiffness-induced intravasation of cancer cells.

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