Therapeutic targeting of the tumor microenvironment

Understanding how tumor and host cells interact to support tumor development and therapeutic resistance

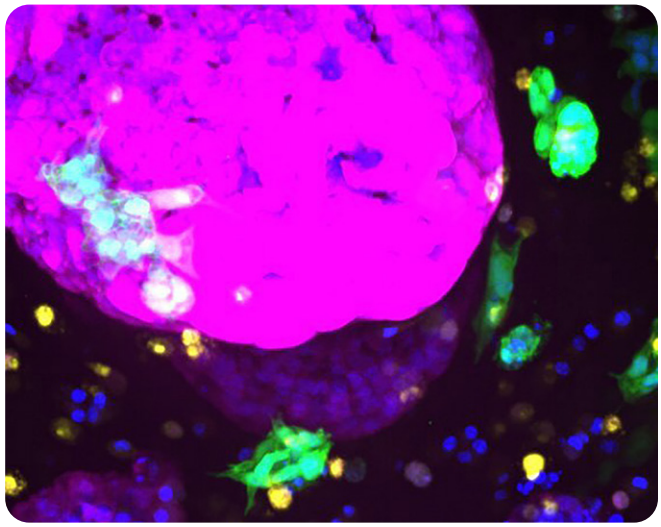
Solid tumours are complex ecosystems encompassing mutated cancer cells along with a variety of non-mutated host cells, such as immune cells, fibroblasts, and endothelial cells. Tumour cells exchange information with host cells to support growth, metabolic shift, and metastasis. Thus, tumour cell function is critically dependent on the local microenvironment; something which needs to be considered when devising therapeutic strategies to overcome resistance.

This paper describes the importance of studying tumor cells in the context of their microenvironment and how 3D models will be important to further address the impact of the microenvironment on tumor cell function and to functionally interrogate stromal targeted therapies in patient-derived models.

The solid tumor microenvironment

Solid tumors develop in complex and continuously evolving microenvironments that can influence their development, behavior, and ultimately progression and metastasis. This tumor microenvironment (TME) comprises a wide variety of cell types and varies between tumor types, but hallmark features include immune cells, stromal cells, blood vessels, and extracellular matrix (ECM).

As tumors evolve, tumor cells and their adjacent microenvironments are in frequent communication. Determining how tumor and host cells interact with one another is a current research focus for Dr. Claus Jørgensen's



lab at Cancer Research UK's Manchester Institute. He believes that understanding the cellular and molecular mechanisms governing these dynamic interactions will be key to improving cancer treatments and overcoming therapeutic resistance. "My interest lies in understanding how these individual subpopulations engage with each other and how these pathways are perturbed in cancer compared to the normal healthy physiological situation," explained Dr Jørgensen. "If we can understand what the TME does and why it is important in solid tumors, we'll probably simultaneously know the best way to attack it from a therapeutic perspective."

A particular interest of Dr. Jørgensen's lab is pancreatic ductal adenocarcinoma (PDA), which is the most common form of pancreatic cancer with an average five-year survival rate of just 9%. In PDA, around 80% of the tumor volume is made up by a stromal component. "It's the poster child of a microenvironment gone bad," he said.

The heterogeneous stroma of PDA consists of immune cells such as macrophages and myeloid cells, mesenchymal stromal cells such as cancer associated fibroblasts (CAFs), as well as ECM, soluble proteins such as cytokines, growth factors, and blood vessels.

The abundance of fibroblast and myeloid cells in the TME of PDA has been shown to reduce immune surveillance and confer resistance to therapy. Furthermore, the extensive deposition and remodeling of the ECM means tumors are typically stiff and avascular. "These tumors are growing in a hostile environment that is not only stiff, but also nutrient deprived and hypoxic," said Dr. Jørgensen, adding that one of the best described ECM modifications is by hyaluronic acid, which acts to retain water and therefore increases interstitial pressure. This in turn results in vascular collapse and poor vascularization.

Modeling the pancreatic cancer microenvironment

In recent years, strategies to target the TME have emerged as a promising approach for therapeutic intervention. Several angles are currently being pursued, including softening the tissue by targeting desmoplasia, decreasing the interstitial pressure and re-vascularizing the tumor to decrease hypoxia, and developing drugs that 'normalize' the microenvironment. "Some beautiful pre-clinical and basic science has been conducted to address these challenges," said Dr. Jørgensen. "However, translating this into the clinic has so far proven very difficult." Recently, Dr. Jørgensen's lab has been working to develop 3D patient-derived models that recapitulate relevant physiological components of the TME in PDA. "In principle, these cell models are a lot easier to establish than your regular 2D cell culture with pancreatic cancer patient samples," he said.

Initially, Dr. Jørgensen's interest in cellular models stemmed from an investigation into the impact of a heterocellular environment on tumor cells. "Our first studies used relatively simple model systems where we co-cultured tumor cells directly with stromal fibroblasts," he explained. "One of the key observations we made was that the effect of oncogenic KRAS, which is the most frequent mutation in PDA, on tumor cell signaling was drastically influenced by stromal fibroblasts." For the team, this finding underscored the importance of understanding tumor cell signaling and function within the context of the TME and led them to explore how tumor and stromal interactions are affected by the addition of other cell types, as well as the impact of tumor cell heterogeneity. "It turned out both aspects are influencing interactions between the tumor and stromal cells," he said. "Moreover, with recent advents in single-cell technologies and mass spectrometry, it became clear that tumors are highly complex with a high degree of both intra- and inter-tumoral heterogeneity."

Developing a 3D model of human pancreatic cancer

Following on from these earlier experiments, Dr. Jørgensen set out to design a fully synthetic 3D model for pancreatic organoids, which would simultaneously capture relevant physiological components of the TME. "Three-dimensional cell models are in principle a lot easier to establish than your regular 2D cell culture with pancreatic cancer patient samples," he said. However, these models typically are grown in basal membrane extract which doesn't allow the stromal cells to grow in a way that the tumor cells would experience within the context of a tumor. "It's not the same and there's a high degree of variability from batch to batch," he said. "We envisaged that an ideal model would replicate essential cell–ECM interactions, mimic tissue stiffness ranges observed across normal and tumor-bearing tissues, support co-culture of epithelial and stromal cells, and facilitate growth and development of organoids directly from tissue samples."

In collaboration with Prof. Martin Humphries, an expert in integrin signaling based at the University of Manchester, and Prof. Linda Griffith, an expert in developing 3D models/scaffolds at MIT Biological Engineering, Dr. Jørgensen set out to develop a model for pancreatic cancer that allowed them to control the growth conditions, co-culture, and tumor stiffness. "The way we went about this was to start by deconstructing the tumor so that we could then start reconstructing the model," he explained.

First, they deconstructed and catalogued the ECM to identify which adhesive substrates were available for cells, and then function tested them to identify essential signals for tumor and stromal cells. "This led us to identify laminin–integrin signaling as being an important factor," said Dr. Jørgensen. In parallel, the team probed the stiffness of the tumors, both across human and mouse tumor models, using atomic force microscopy. "We learnt just how widespread variation there was in the stiffening of these tumors," he said.

With these pieces of information, they reassembled 3D models based on available reagents to mimic adhesive signals, organoids, tuning of stiffening, and co-culture and ended up with a model that supported human and mouse organoids, stromal co-culture, and that could be tuned to recapitulate the entire stiffness range of these tumors.[1]

Dr. Jørgensen noted that working with such complex models presented numerous challenges, from selecting and optimizing growth medium and conditions for co-culture to successfully presenting the adhesive peptides. "Stromal and immune cells have different requirement for adhesive signals and growth medium/growth factors, which is something that we started to address but that needs more work to include additional cells from the TME," he explained. "While this presents a challenge for day-to-day use, it also presents an opportunity to learn something fundamentally important. Because these cells co-exist within a tumour, we know that given the correct cocktail of signals/cell populations and their state, we should be able to re-establish the multicellular models *in vitro* without much additional growth supplement. In doing so we will know that the cellular equilibrium *in vitro* likely reflects at least an essence of the *in vivo* experiment."

Future plans

From a therapeutic perspective, Dr. Jørgensen hopes that these models could help close the gap between what is seen in the preclinical settling and translation into patient benefits. "By embedding these organoids in a model that is more physiologically relevant, you can start teasing out additional parameters that might be important for how we actually treat these patients," he said. In collaboration with Prof. Humphries and Griffith, Dr. Jørgensen plans to incorporate additional cell models to explore factors such as rigidity signalling and tumour cell response to different stromal co-culture conditions. "One aspect that would also be fascinating to follow up on is the impact of rigidity within the stroma and whether/how this further regulate stromal phenotype."

Claus Jørgensen

Dr. Claus Jørgensen obtained his BSc in Biochemistry and Molecular Biology at the University of Southern Denmark, Odense Denmark in 1999. At the same University, he later received his PhD in 2005. His Post-Doctoral position took him to Toronto, Canada, and the Samuel Lunenfeld Research Institute, under the guidance of Professor Tony Pawson. It was during his four-year training with Prof Pawson that he developed a keen interest in reciprocal signaling. In 2010, Claus moved to The Institute of Cancer Research, London, to lead the Cell Communication Team. Here, he focused on using and further developing an experimental platform to interrogate heterotypic cell signaling and its impact on tumor formation and progression. In 2011, Claus was awarded a CRUK Career Development Award. He joined the CRUK Manchester Institute in early 2014 to form the Systems Oncology group, which focuses on the complex interactions between malignant and normal cells, with a particular interest in pancreatic cancer. In 2017 Claus was awarded an ERC Consolidator Grant.

Reference

1. https://doi.org/10.1038/s41563-021-01085-1

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com