A SPARC of originality

Professor Rolf Brekken is interested in a protein known as SPARC. Here, he describes the goals of his research, his team's unique approach and two of the critical molecules in their work

Where does the secreted protein acidic and rich in cysteine (SPARC) protein originate? What leads to its expression?

SPARC is secreted from cells. Virtually any cell can produce SPARC. In fact most cells that are adherent in tissue culture produce SPARC at a detectable level. Growth of cells in culture is stressful and SPARC is a protein that is often produced by cells under stress, thus the concept of it as a 'culture shock' protein was established. In tissues, cells that are responsible for or responsive to tissue remodelling make SPARC. Fibroblasts and macrophages are major producers of SPARC. Endothelial cells are also a significant source during angiogenesis. Tumour cells can produce SPARC although expression is suppressed in many epithelial derived tumours by promoter hypermethylation.

How does your work differ from previous studies in your field?

We are striving to provide a mechanistic explanation for SPARC function in the context of the tumour microenvironment. SPARC has been shown to impact many different pathways *in vitro* and to contribute to multiple biological events *in vivo*, yet how it functions is largely unclear. We have shown that it directly affects activation of latent transforming growth factor beta (TGFB) and this work provided significant mechanistic insight into how SPARC reduces TGFB signalling; however, a validated global hypothesis for SPARC function is not available.

Given the importance of SPARC for collagen deposition and the abundance of collagen in vertebrates, we propose that its main function is centred on collagen biology. Thus, over the last few years we have focused on trying to understand how SPARC might affect collagen biology. Much of this work has been done in collaboration with Dr Amy Bradshaw at the Medical University of South Carolina. The Bradshaw lab has demonstrated that SPARC reduces collagen binding to the surface of cells and cells deficient in SPARC have elevated levels of surface-associated collagen.

Could you define TGFB? How do secreted glycoproteins regulate TGFB signalling?

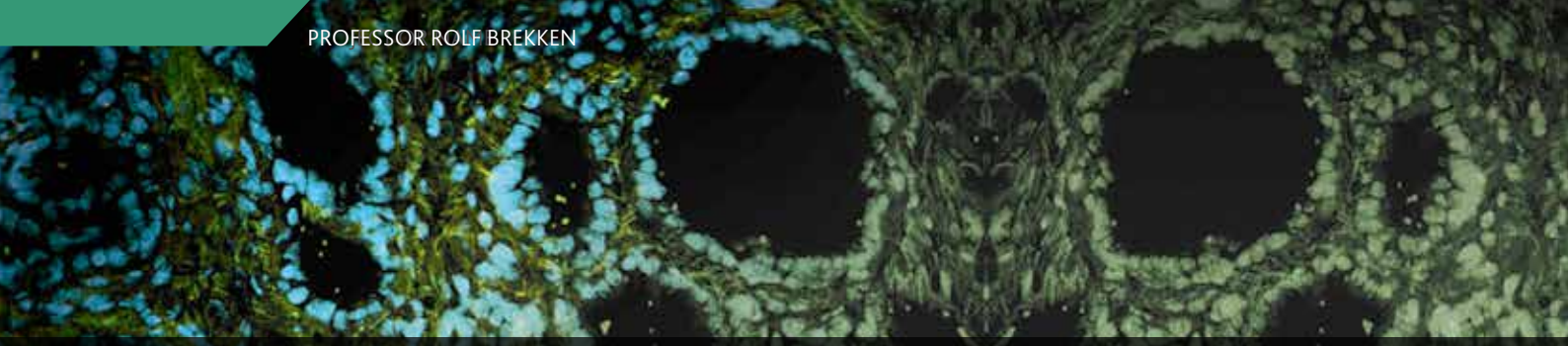
TGFB is a multifunctional cytokine that participates in many biological processes. We are particularly interested in TGFB function during angiogenesis and tumour development and progression. During vascular remodelling TGFB can have multiple functions based on how much and when the protein is expressed and activated. The protein is secreted in an inactive form that must be 'switched on' before it can bind its receptors to activate the canonical TGFB signalling cascade. TGFB biology is complex and is regulated at multiple levels. We demonstrated recently that SPARC slows the activation of latent TGFB on the surface of pericytes. We also identified that pancreatic tumours grown in SPARC-/- animals have elevated levels of active TGFB that contribute significantly to accelerated disease progression.

What is your long-term goal for this research? How will this be realised?

The level of scientific interest in extra-cellular matrix (ECM) proteins and matricellular proteins is not as high as it should be and is certainly not on par with their importance to tumour biology. I hope that our studies can help change that. In addition, we do have specific goals for each project. For example, for the Fibulin-5 (Fbln5) project we are developing inhibitors of Fbln5 which we hope could in the future be used as therapeutic agents. For the SPARC project we hope to document that collagen signalling is an important aspect of tumour biology. There are inhibitors available (eg. imatinib, nilotinib) that reduce collagen signalling and these or other agents might be useful to combat collagen-induced chemoresistance.

Finally, what other aspects of research are you currently engaged in?

Another major aspect of my laboratory's work is centred on VEGF biology in tumours. In particular I have spent the better part of 20 years studying anti-VEGF therapy. I have always thought that the ECM side of the lab would be distinct from the anti-VEGF side; however, these two research directions are growing together in a very organic way. We and many other groups have found that anti-VEGF therapy reduces microvessel density in tumours and as a result increases hypoxia. In fact hypoxia turns out to increase the expression of TGFB, which elevates collagen expression and deposition and we see this in tumours from mice treated with anti-VEGF monoclonal antibodies. TGFB also induces epithelial to mesenchymal transition (EMT), a process that occurs much more efficiently when cells are on a collagen matrix. Therefore a major question we are beginning to address in the lab is whether collagen signalling is critical to anti-VEGF induced EMT and if tumour cell-derived SPARC affects this process.



Probing the matrices of tumour tissue

Researchers at the **University of Texas Southwestern Medical Center** are interested in cells and their environment, looking at chemo-resistant cancers. Their current research focuses on the regulation of tumour development – specifically those of the pancreas – by molecules from the extra-cellular matrix

PANCREATIC CANCER IS the fourth leading cause of cancer-related death in the Western world, affecting thousands of people every year. It is a particularly cruel cancer due to its poor prognosis – with five-year survival estimates as low as 5 per cent. To make matters worse pancreatic cancer can be highly chemoresistant and unresponsive to otherwise aggressive drugs which unfortunately have a range of side effects that further detract from patient quality of life. For these reasons, pancreatic cancer is a disease that requires novel therapeutic approaches which stem from basic research.

Research into the development of tumours often focuses on cellular and genetic mechanisms. After all, mutations in DNA cause the majority of cancers (others can be caused by a virus, for example cervical cancer). However, these mechanisms are only one aspect of the complicated function of tumours, which are tissues that consist of multiple cell types and extracellular components. For this reason Professor Rolf Brekken and his colleagues from the University of Texas Southwestern Medical Center are studying the pathways and important active molecules in the extra-cellular matrix (ECM) of tumour tissue.

The ECM is essentially the space between cells, but far from being empty it is filled with important polysaccharides and proteins which perform a vast range of functions, including inter-cellular communication, structural support and molecular recognition. The cells that exist within the local ECM are responsible for its composition: "The ECM is produced, maintained, altered and remodelled by the cells that reside within it," explains Brekken. Similar to the cells responsible for the ECM, the matrix itself is in constant flux, responding to the relevant biochemical pathways and signals it receives. Two important molecules which function in the ECM are secreted protein acidic and rich in cysteine (SPARC) and fibulin-5 (Fbln5). Brekken and his team have focused on understanding the impact and effects that these molecules have in the process of tumour formation with the ultimate view of improving existing treatments or developing new therapies, with efforts so far focused on pancreatic cancers.

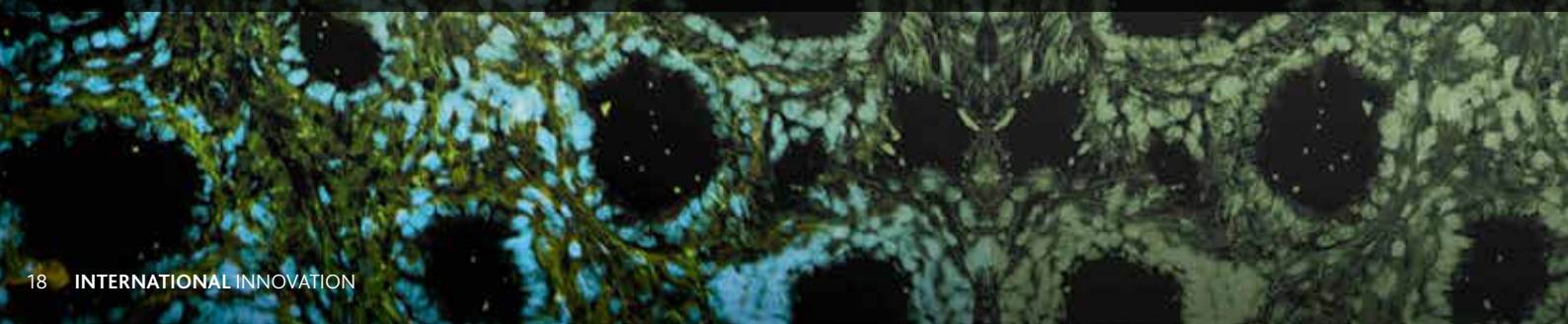
SPARC OF HOPE

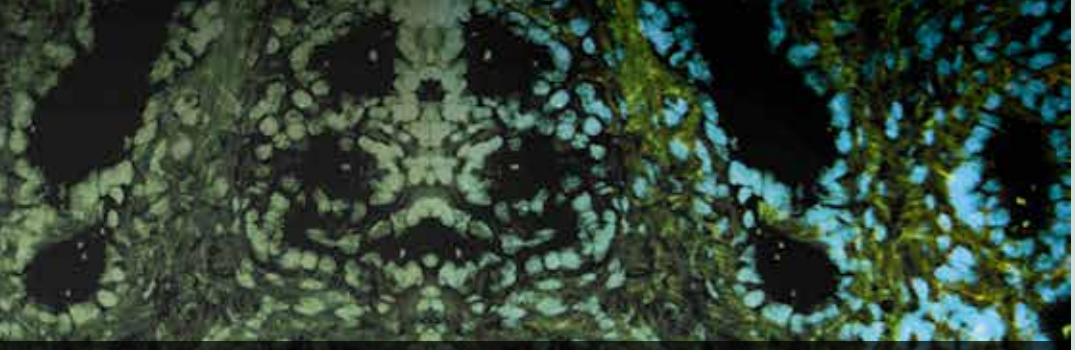
The researchers have already enjoyed notable success in this pursuit, identifying an important and dramatic difference in tumour development between wild-type (normal) and SPARC-deficient mice. They observed that mice engineered to be incapable of producing SPARC were subjected to far more aggressive tumours than their wild-type counterparts. The aggressive pancreatic tumours in the SPARC-deficient mice unsurprisingly led to a shorter life span. On top of this, in the absence of SPARC the aggressive tumours contained fewer blood vessels (a 20 per cent reduction in micro-vessel density). Importantly, this tells us that SPARC is a central player in the regulation of tumour development. These relatively early discoveries led to the formation of one of Brekken's primary research goals: "The main goal of this part of my laboratory is to demonstrate how matricellular proteins, such as SPARC and Fbln5 contribute to tumour development and progression".

Brekken and colleagues hypothesise that the dominant function of SPARC is the regulation of collagen deposition and signalling. They are now using SPARC-deficient mice to demonstrate that collagen signalling is increased when the regulatory molecule is absent. This line of research has naturally led to questions regarding the mechanism of collagen deposition on tumour growth, as Brekken elaborates: "We are now investigating collagen signalling and how it contributes to tumour progression through induction of epithelial to mesenchymal transition (EMT) and chemoresistance".

OXIDATIVE STRESS – FBLN5

SPARC is not the only ECM-related protein the Brekken lab studies – the team similarly focus on a second matricellular protein called Fbln5. This molecule is known to regulate elastic fibre formation and fibronectin mediated cell signalling. Similarly to SPARC, Brekken and colleagues have engineered Fbln5-deficient models to assess the function of the protein in the tumour microenvironment. The absence of Fbln5 in models of pancreatic cancer





resulted in a significant reduction in tumour growth and induced an increase in the level of reactive oxygen species (ROS) in the tumour microenvironment. Specifically, the Brekken lab identified that in the absence of Fbln5 there is an increase in fibronectin mediated cell signalling that results in elevated ROS in vascular cells. Armed with this new knowledge the team is now in the process of developing a compound capable of inhibiting Fbln5, thus producing a similar increase in ROS within the tumour environment and a subsequent decrease in invasive growth.

ECM proteins contribute to chemoresistance in pancreatic cancer and potentially other disease sites will hopefully contribute to improved therapeutic strategies.

With notable success up until now, it is vital for the researchers to maintain momentum and drive forward. In this regard the group has set a wide range of new and developing objectives, including an attempt to understand how SPARC affects chemoresistance in pancreatic tumours. This work has recently enjoyed some publicity

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While Brekken and his team are moving forward rapidly with their research they are aware of the need for caution. The work so far has produced compelling and impressive results however the data is based on animal models, and primarily mice: "We cannot be certain that our studies on SPARC and Fbln5 will translate faithfully to human cancer patients," admits Brekken. Despite this limitation the research will allow them to look for pathway biomarkers which would indicate similar processes in human tissue.

CHOOSING CHEMORESISTANT CANCER

Chemoresistance is a particular issue in cancers of the breast and pancreas, which are often resilient to traditional therapies. While this property makes these and similar cancers particularly problematic to treat, these tumour types make ideal study subjects: "Pancreatic cancer in particular is a good setting to study SPARC biology because this disease is desmoplastic, highly metastatic and chemoresistant," he points out. Understanding the mechanism(s) of how as SPARC has been implicated as a biomarker in the response to chemotherapy. As mentioned, the work with Fbln5 has led to the development of potential candidate molecules to block its action and thus increase ROS mediated tumour stress. The team hopes that these future drugs can become a tool to oxidatively stress cancerous cells, perhaps in cooperation with other anti-cancer agents.

By understanding the pathways involved in how the ECM contributes to the tumour environment Brekken and his colleagues hope to positively impact human disease either through the development of better therapeutic agents or through the exploitation of the characteristics of the tumour microenvironment. The group's research has already contributed significantly to knowledge of tumour development and may soon add to the list of tools available to combat tumour development. The studies thus far have also highlighted the importance of broadening collective research efforts that can exploit the biology of the cellular and extracellular components that contribute to tumour progression.

INTELLIGENCE

MATRICELLULAR PROTEINS AS REGULATORS OF TUMOUR PROGRESSION

OBJECTIVES

To develop improved strategies for the therapy of pancreatic cancer by defining and exploiting the function of SPARC and Fibulin-5 in the tumour microenvironment.

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ROLF BREKKEN received his BA in Biology from Luther College in Decorah, Iowa and his PhD from UT Southwestern Medical Center where he developed therapies that target the vascular compartment of tumours. His postdoctoral studies at the Hope Heart Institute in Seattle, Washington were focused on how SPARC contributes to vascular function in tumours. He is currently the Effie Marie Cain Scholar in Angiogenesis Research and an Professor in the Department of Surgery at UT Southwestern and his laboratory is located in the Hamon Center for Therapeutic Oncology Research where his group studies the tumour microenvironment.

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