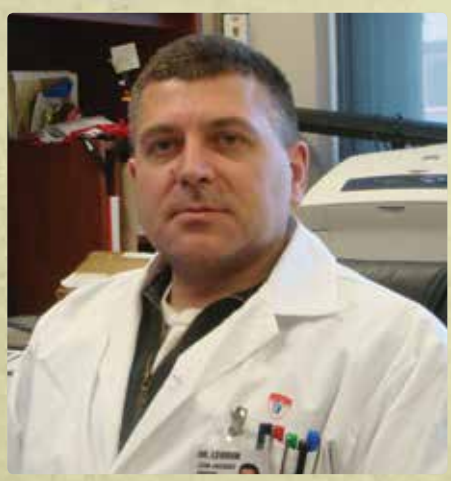
DR JEAN-JACQUES LEBRUN

A difficult duality

Dr Jean-Jacques Lebrun's investigation into the dual roles of TGFβ in cancer will enable the development of novel therapeutics targeting the signalling mechanisms underlying the disease



Could you begin by outlining the main research objectives of your laboratory?

Our various projects are based on the dual role of transforming growth factor beta (TGFβ) in cancer. TGFβ exerts tumour suppressive effects in normal cells and in early carcinomas, whereas in advanced cancer it exerts pro-migratory and pro-invasive effects. Therefore, we have developed two lines of research: 1) defining the tumour suppressive role of TGFβ – how this growth factor inhibits cell proliferation, induces apoptosis and prevents cell immortalisation in normal cells and early carcinomas; and 2) defining the pro-metastatic effect of this cytokine in invasive cancers, focusing on breast cancer with the goal of designing novel anti-TGFβ signalling therapies.

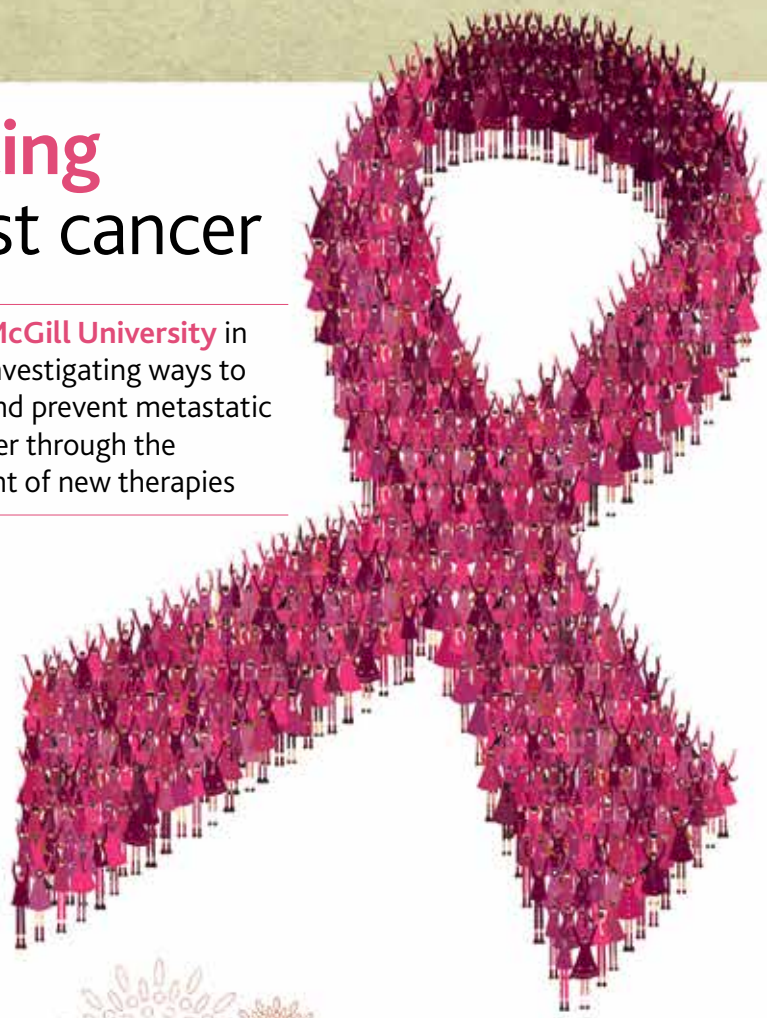
Your 2002 study was the first to link phospholipid metabolism to TGF-β-mediated apoptosis. What impact has this had on subsequent research?

We found that TGFβ could induce apoptosis in haematopoietic cells through expression of the inositol phosphatase SHIP, a central regulator of phospholipid metabolism. Induced expression of SHIP leads to a change in the intracellular pool of phospholipids and inhibition of the phospholipid-dependent protein kinase AKT, prompting apoptosis. Thus this seminal study linked, for the first time, phospholipid metabolism with TGFβ-mediated apoptosis.

We explored the molecular mechanisms by which TGFβ induced cell death in other tissues and tumour types, and found that transcription

Beating breast cancer

A team at **McGill University** in Canada is investigating ways to pre-empt and prevent metastatic breast cancer through the development of new therapies



TRANSFORMING GROWTH FACTOR-BETA (TGFB) is a near-ubiquitous cytokine responsible for the regulation of cell growth, and division and differentiation in all types of tissue. It also plays a major role in the determination of cell fate during early development and embryogenesis. TGFβ is involved in hormonal and immune responses, cell growth, cell death and immortalisation, and the repair and remodelling of tissues throughout adult life. The biological and physiological functions of this cytokine and its receptors are central to human disease, with perhaps the most notable example being cancer; a significant number of human tumours result from mutations or deletions in genes which code various TGFβ signalling components.

Cancer mortality rates have recently risen above those for cardiovascular disease and are now higher than for any other disorder, making the need for novel and effective treatments more pressing than ever before. In 2012, 1.8 million new cases of cancer were diagnosed in the US and Canada, including 250,000 cases of breast cancer. The most common form of cancer affecting women in North America, breast cancer is estimated to have claimed the lives of some 45,000 individuals in that year alone. While remission and overall survival rates

factor E2F1 is central to the mediation of these effects, while TGFβ leads to the formation of a transcriptionally-active E2F1-pRb-P/CAF complex on the TGFβ pro-apoptotic target gene promoters, activating their transcription. These findings defined a novel process of gene activation by the TGFβ-E2F1 signalling axis, showed E2F1 as a central mediator of the TGFβ apoptotic programme and highlighted the pRb-E2F1-P/CAF pathway as a wide-ranging and critical mediator of cell death in multiple target tissues.

What techniques and methodologies did you use to reveal new pathways, involving p21 and cyclin D1 proteins, activated downstream of TGFβ in breast cancer?

For the *in vitro* studies, mRNA and protein expressions were assessed by quantitative real-time polymerase chain reaction and western blot. Co-localisation and interaction between cyclin D1 and p21 were performed by immunocytochemistry and co-immunoprecipitation, respectively. Cellular invasion was measured using Transwell Matrigel assay. The effects of cyclin D1 on cellular structure and actin organisation were examined by staining with F-actin marker phalloidin and mesenchymal intermediate filament vimentin. *In vivo*, mammary fat pad xenograft and intratibia injection mouse models were used to assess the mammary tumour growth, local invasion and the development of bone osteolytic lesions. The association between active TGFβ/p21/Cyclin signalling with lymph node metastasis was examined by immunohistochemistry in tissue microarray containing 50 invasive ductal breast tumours, half of them being lymph node-positive.

Could you provide more detail on the roles of p21 and cyclin D1? How is their activity affected by TGFβ?

A first study unveiled an unexpected and critical role for p21 in breast cancer progression – TGFβ increasing p21 expression – leading to tumour cell invasion and migration. TGFβ-induced p21 association with the acetyltransferase P/CAF also led to activation of gene transcription of a subset of genes critical for the invasive process. We subsequently found that TGFβ could induce expression of cyclin D1 in triple negative breast cancer cells, resulting in TGFβ-mediated cell migration. We also discovered that TGFβ promotes nuclear co-localisation and physical interaction between cyclin D1 and p21. The co-expression of cyclin D1 and p21 proteins is required for the initial steps of tumour development, as double knockdown of these molecules prevents primary tumour formation and local tumour invasion in a xenograft mouse model.

You characterised a new TGFβ signalling route involving micro-RNA (miRNAs). Could you explain how this affects breast cancer cell migration?

Recently, it has become clear that miRNAs, a new class of small regulatory molecules, play crucial roles in mediating tumour formation and progression, and we became interested in investigating whether some of the pro-metastatic functions of TGFβ involved miRNAs. Our research showed that miRNA-584 (mir-584) acts downstream of TGFβ in the regulation of the metastatic process, and TGFβ downregulates the expression of miR-584 in breast cancer cells. After identifying that PHACTR1, an actin-binding protein, is positively regulated by TGFβ in a miR584-dependent manner, we also found that TGFβ-mediated downregulation of miR-584 and PHACTR1 increased expression are necessary for TGFβ-induced cell migration of breast cancer cells. Our study defined a novel signalling route whereby TGFβ silences the expression of the miRNA, miR-584, enhancing expression of the actin-binding protein PHACTR1 and further leading to actin re-arrangement and breast cancer cell migration.

are high in cases of localised primary tumours, they are dramatically lower for aggressive tumours which have propagated to distant sites. These metastatic tumours are responsible for the majority of breast cancer-related deaths, and currently no effective treatment exists. TGFβ, insofar as it plays a role in inhibiting cell proliferation, preventing cell immortalisation and inducing apoptosis, can have a potent tumour-suppressant effect in both healthy cells and early carcinomas.

SWITCHING SIGNALS

Dr Jean-Jacques Lebrun is Associate Professor of Medicine and the former Director of the Hormones and Cancer Research Unit at McGill University in Montreal. He coordinates a laboratory investigating the effects of transforming growth factor-β (TGFβ) signal transduction in cancer formation and progression. His team's primary research goal is to investigate the role and mechanism of action of growth factors from the TGFβ family in the regulation of tumour formation and cancer progression, with a long-term aim of developing novel therapies against metastatic cancer. With an emphasis on the molecular mechanisms by which TGFβ signalling regulates cell growth and apoptosis in aggressive breast cancer, the investigators have identified several new important TGFβ target genes and downstream signalling pathways which play a major role in mediating the growth factor's tumour-suppressant effects. "Our research demonstrates that TGFβ family members regulate growth inhibition, apoptosis and immortalisation in human cancer cells of various origins," Lebrun outlines, "including breast cancer, hepatocarcinomas, colon cancer, blood-born cancers and pituitary tumours."

As tumours develop, the protective tumour-suppressive effects of TGFβ are lost and its signalling changes, switching instead to promoting cancer progression and tumour metastasis, rather than inhibiting them. The surrounding extracellular matrix, which acts as a TGFβ reservoir, is remodelled and degraded, releasing its stored TGFβ which then exerts autocrine effects on the tumour cells, as well as paracrine effects on components of the tumour milieu, including stromal fibroblasts, endothelial cells and immune cells. The cancer cells respond to, and are affected by, the increased TGFβ levels, undergoing phenotypic change from epithelial to mesenchymal – a loss of polarity and adhesion associated with increased migration and invasive properties. There is also increased chemoattraction to distant tissues, intensifying the chances of the development of metastatic disease. Paracrine effects include the stimulation of angiogenesis, contributing to myofibroblast differentiation and causing local and systemic immunosuppression, which in turn further encourage tumour progression and metastasis.

TRIPLE NEGATIVE

Lebrun's research includes the study of triple negative breast cancer (TNBC), which has a poor prognosis and a high risk of tumour recurrence. Although they only constitute some 10-20 per cent of tumours, TNBCs are responsible for the vast majority of breast cancer-related deaths. More aggressive and invasive than hormone-sensitive breast cancer, there is also no effective treatment for these tumours. Understanding and elucidating the molecular mechanisms behind their formation and progression is therefore of vital importance in the development of new therapeutic approaches to breast cancer, and the team has already made a significant finding which opens up new avenues for new, anti-metastatic breast cancer therapies.

The project employs both *in vitro* and *in vivo* methods in its investigation of TNBC, utilising

NOVEL FUNCTIONS FOR P21 AND CYCLIND1 IN TGFB-MEDIATED BREAST CANCER METASTASIS

OBJECTIVES

To understand the molecular mechanisms underlying tumour formation and metastasis, with the goal of developing novel drugs with anti-metastatic properties.

KEY COLLABORATORS

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several human breast cancer cell lines issued from different patients with metastatic breast tumours, alongside a breast cancer database of 761 patients and breast cancer tissue microarray analysis of 50 patients. "Our investigations unveiled a novel pathway downstream of TGFβ that leads to tumour progression," Lebrun reveals, "and showed that blocking expression of p21 and cyclin D1 through small molecule RNA interference efficiently prevented local tumour invasion of breast cancer cells to the surrounding mammary epithelial tissues."

CANCER STEM CELLS

The lab has also made inroads into understanding cancer stem cells (CSCs) – small populations of tumorigenic cells which exist within each tumour and exhibit properties similar to embryonic stem cells. They are able to self-renew and differentiate, giving rise to new tumours and driving metastasis. They are also resistant to radiation and chemotherapy, causing local and distant tumour relapse and increasing patient mortality rates. Accordingly, these cells constitute a key target for new therapeutics. "There is a clear need for novel therapeutic regimens that target these CSCs," asserts Lebrun. "The aggressive properties of basal-like triple negative breast cancers are partly due to the presence of high proportions of cancer stem cells within the TNBC tumours."

> Cancer mortality rates are now higher than for any other disease, making the need for novel and effective treatments more pressing than ever

The McGill University researchers are currently investigating the role of TGFβ ligands in regulating cancer stemness and self-regeneration in TNBCs, and the findings to date indicate that TGFβ increases the proportion of the highly tumorigenic CD44high/CD24low/EpCAM+ cancer stem cell-like phenotype, promoting their capacity for self-renewal. The team is now investigating the molecular mechanisms by which TGFβ regulates CSC self-renewal and differentiation in human breast cancers. They hope that, in time, this work will facilitate the development of targeted treatments which will disable the self-renewing capabilities of CSCs, preventing tumour propagation and metastasis.

LONG-TERM GOALS

Apart from the discovery of key molecules, such as p21 and cyclin D1, which play a central role in promoting tumour cell invasion and tumour metastasis, Lebrun and his colleagues have also found that using small hairpin RNAs (shRNAs) to block the expression of these molecules – a method known as RNA interference – efficiently delays or prevents tumour cell local invasion *in vitro* and *in vivo*. Their next steps include investigating whether and how shRNAs or similar derived products might be efficiently delivered to breast cancer patients, while using clinical trials to assess their efficacy in preventing the propagation of metastatic disease.

Continuous advances in the field are leading to an ever-more detailed understanding of the mechanisms underlying tumour formation and progression, and Lebrun hopes that in the future, his team will be able to counteract the specific signalling pathways crucial to the progression of the disease and the formation of metastases. "Elucidation of the key components driving these effects, such as p21 and cyclin D1, will provide effective new treatments aiming at inhibiting local tumour cell invasion and cancer stem cells (CSC) self-renewal," he concludes, "as well as eliminating CSC populations in distant metastasis breast cancer patients."

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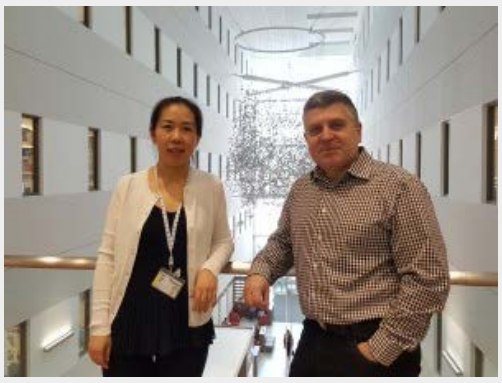

Dr. Meiou Dai and Dr. Jean-Jacques Lebrun (Photo: Nadege Fils-Aime, PhD student in the lab of Jean-Jacques Lebrun)

Triple negative breast cancer (TNBC) is the most aggressive type of breast cancer, found in 15-20% of all cases. These breast tumors are enriched in a rare population of cancer cells, called cancer stem cells, which are responsible for the high rates of metastasis, resistance to chemotherapy and tumor recurrence. There are very few biomarkers for this cancer type and, there are no proven treatment options to combat it.

New hope is on the horizon, thanks in part to research led by senior author Dr. Jean-Jacques Lebrun, Professor of Medicine at the McGill University Health Centre Cancer Research Program. In the study, published in *Nature Scientific Reports*, Dr. Lebrun, working with McGill colleagues Dr. Suhad Ali and Dr. Meiou Dai, found that the expression of cyclin-dependent kinase (CDK4) gene was higher in breast cancer tumours when compared to normal tissues, and was at the highest level in aggressive tumours of the TNBC subtype. They also identified CDK4 to serve as a potential therapeutic target to combat TNBC tumors.

“Our study highlights new roles for CDK4 in regulating cancer stemness and as a novel prognostic marker in TNBC,” says Dr. Lebrun. Because the TNBC tumours are enriched with cancer stem cells – cells that utilize their self-renewal ability to generate and persistently propagate a heterogeneous tumor which results in metastasis and cancer relapse, while also displaying resistance to chemotherapy causing cancer patient death – targeting these cancer stem cells is a priority for the development of novel therapies that could efficiently target TNBCs.

CDK4 and its binding partner cyclin D1 are the ultimate targets of many oncogenic signals, suggesting a central role for these proteins in cancer development and progression. “We previously found that cyclin D1, a CDK4 binding partner, functions as downstream of TGFβ signaling pathway to regulate metastatic processes including cell migration and invasion of TNBC” explains Dr. Dai, the study’s first author. “This prompted us to then investigate the potential role played by the cyclin D1 partner, CDK4 in breast cancer progression. We performed a bioinformatics analysis of CDK4 in breast cancer, and interestingly, we found that CDK4 expressed at the highest level in TNBC tumors and correlated with poor prognostic and early relapse-free survival outcome.”

Using the Pfizer CDK4 inhibitor “Palbociclib”, which is FDA approved in combination treatment with aromatase inhibitors for hormone-responsive breast cancer and is currently in clinical trial, the researchers discovered new functions for CDK4 in regulating cancer stem cell differentiation and chemotherapy drug resistance in TNBCs.

“We found that blocking CDK4, using Palbociclib prevented breast cancer stem cell self-renewal and efficiently eliminated cancer stem cells as well as chemotherapy-resistant cancer cells, highlighting CDK4 as a promising targeted therapy for these aggressive breast tumors for which no effective treatment currently exists,” notes Dr. Lebrun.

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