Could you provide a brief overview of your research, which investigates circulating tumour cells (CTCs) in the bloodstream of prostate cancer patients? Why are you focusing on prostate cancer in particular?

Prostate cancer is a leading cause of cancer incidence and death in Canadian men. However, the majority of deaths do not occur as a result of the primary prostate tumour, but rather from the spread of cancer cells to other vital organs. This process is known as metastasis, and the tumours formed in other organs are called metastases. CTCs have been shown to be useful in predicting patient outcomes in metastatic prostate cancer using the current gold standard CTC enumeration technology, the CellSearch® system. This platform not only provides a minimally invasive means of patient sample collection and CTC enumeration, but also allows for limited molecular characterisation of CTCs.

Characterisation of isolated CTCs could lead to a better understanding of the biological mechanisms that underlie metastasis, aid in the identification of novel targets for new therapies, and ultimately help to direct patient care. In addition to exploring the molecular characteristics of CTCs, we also aim to investigate several areas of research that have been largely unexplored, including the potential role of CTCs in early-stage disease and therapy response, as well as exploiting an in vivo model system of prostate cancer to gain a better understanding of the biology of CTCs and their contribution to the metastatic cascade.

What is the importance of fully understanding the metastatic cascade?

Almost 40 per cent of women and 45 per cent of men in Canada will develop cancer during their lifetime, and one in four will die from the disease. Strategies for treating early-stage, localised disease are highly successful; however, few treatment options exist for patients with metastatic disease. The ineffective treatment of this stage of disease is due, in large part, to a general lack of understanding of the metastatic cascade. Therefore, research is necessary to address the basic biology of the cascade, develop drugs or treatments targeting it, as well as further the development and improvement of techniques to track disease progression and strategies to prevent metastasis. Ultimately, we anticipate that a greater understanding of metastatic disease will lead to improved patient outcomes and overall survival.

Are there any challenges in the field of CTC research that your lab is currently addressing?

CellSearch® has been utilised clinically to effectively detect CTCs in the blood of metastatic breast, prostate and colorectal cancer patients. However, CTCs are undetectable in up to 35 per cent of patients with various metastatic cancers, despite the disease being widespread. This lack of detection is thought to be due to the epithelial-to-mesenchymal transition (EMT), a process in which cells become invasive and lose expression of the epithelial markers necessary for their detection using CellSearch®. This suggests that the standard CellSearch®
Three characteristics define malignancy in a tumour: invasiveness, anaplasia – or loss of cellular differentiation – and metastasis, but it is this last trait that often makes cancer such a feared disease. In part, this is because current treatments for primary disease are far more effective than those available for metastatic disease. Prostate and breast cancer, for instance, are the two most common causes of cancer death in Canada – as in most of the developed world – and yet primary tumours only account for a very small number of these fatalities.

The metastasis of a tumour is a relatively simple process, and occurs when cells from the primary tumour enter the circulatory or lymphatic systems. Tumour cells found in the circulation are referred to as circulating tumour cells (CTCs), and when these cells reach a suitable part of the body, they may ‘seed’ a new tumour. CTCs were first identified in the mid-19th Century, and have long been recognised as an integral component of the metastatic process. It is only recently, however, that scientists have had access to the technology required to begin searching for rare CTCs in the blood of patients. This facility opens up the possibility of looking at CTCs as windows into the metastatic process, effectively presenting a non-invasive alternative to biopsy collection of metastatic tumours, as this is not always a possibility.

Researchers at Western University in Ontario, Canada, are working on a project that they hope will improve methods for detecting and characterising circulating tumour cells – the cells primarily responsible for metastasis in prostate cancer. Having the ability to predict that cancer has spread from the primary site earlier than currently available technologies is advantageous, but there is one problem. In as many as 35 per cent of metastatic cases, CTCs have been found to be undetectable in the bloodstream, despite an obvious presence of widespread disease. This means that there are two possibilities: either around one-third of patients with metastatic cancer genuinely have no CTCs in their system, or the CTC detection methods currently being used are employing a definition of CTCs that do not reflect the entire population.

One laboratory at Western University believes that CTCs are likely to be found in the bloodstream of all patients with metastatic cancer and also happens to have access to one of Canada’s six CellSearch® systems, the gold standard for CTC detection. PhD candidate Lori Lowes works as part of Dr Alison Allan’s group in the Department of Anatomy and Cell Biology, and for some time its aim has been to improve methodologies for enumerating and characterising CTCs. Making use of their state-of-the-art CellSearch® platform, which is amenable to the development of user-defined protein marker protocols as well as having the capability to enumerate CTCs using a standardised method, the researchers hope to...
MOLECULAR CHARACTERIZATION OF CIRCULATING TUMOR CELLS IN PROSTATE CANCER

OBJECTIVES

• To examine and identify the limitations of existing circulating tumour cell methodologies
• To examine the metastatic cascade, its mechanisms and the molecular characteristics of the cells involved
• To examine the relationship between therapy responses, circulating tumour cell detection and molecular characteristics, with the potential for the identification of novel targets for new therapeutic drugs
• To better understand and utilise circulating tumour cells as a means to improve patient care and overall survival

KEY COLLABORATORS

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ULTIMATELY DEVELOP CTCs INTO A BETTER TOOL FOR IMPROVING PATIENT CARE AND OVERALL SURVIVAL

TRACKING DOWN TUMOURS

Beyond the technical aspects of their work, Lowes and her colleagues are interested in the limitations of current CTC approaches, as well as studying the metastatic cascade, its mechanisms and the molecular characteristics of the cells involved. The Ontario lab also aims to improve current understanding of the relationship between therapy response, CTC detection and molecular characteristics. Over the last five years, therefore, the researchers have been involved in a number of varied projects making use of both rodent models and prostate cancer patient cohorts – and in some instances, their results have been quite surprising.

In a 2011 study, the group showed that CTCs could be a valuable tool for clinical decision-making when it came to salvage radiotherapy. 30 per cent of prostate cancer patients will experience a recurrence of cancer within 10 years of radical prostatectomy, requiring radiotherapy – but using current clinical technology, there is no way of knowing whether the recurrent cancer will be local to the prostate bed or distant from it. As a result, a cohort of these patients will receive radiotherapy treatment that will have no benefit. The Ontario group believes that CTC enumeration could be a more effective prognostic cancer tool as it is more sensitive than current techniques for the early detection of metastatic spread and the researchers are currently confirming their findings in a second, larger study.

In as many as 35 per cent of metastatic cases, circulating tumour cells have been found to be undetectable in the bloodstream

MORE MOLECULAR MARKERS

More recently, the scientists have published a technical manuscript detailing work towards the development of user-defined protein marker protocols for CTCs using the CellSearch® system, which may be of use to other laboratories with access to the same equipment. The hypothesis behind this work is that the molecular characterisation of CTCs may be useful in predicting the response of patients to therapy. In this study, the team developed protocols for two novel markers: the apoptosis marker M-30 and the cancer stem cell marker CD44, an indicator of aggressive disease. It was found to be unsuitable, with CellSearch® only capable of detecting 60 per cent of the expected number of apoptotic tumour cells after paclitaxel treatment. Using CD44, however, the researchers were able to develop a protocol capable of detecting nearly 100 per cent of the expected number of CD44 positive cells using CellSearch®. In 2013, the group also published a set of three protocols for the information of other CellSearch® users, outlining the processes for standardised CTC enumeration, customised molecular characterisation, as well as enumeration in rodent models.

FIGHTING THE FUTURE

CTCs are extremely rare and require incredibly sensitive technology such as CellSearch® for their detection and enumeration, however the additional insights they are capable of providing clinicians with have the capacity not only to reduce the number of unnecessary or inappropriate treatments for cancer, but also to improve survival rates. Ultimately, however, this technology can only be put to good use if it is first optimised and made accessible by frontline researchers such as those in Lowes’ lab.

CELLSEARCH® SYSTEM

LORI LOWES’ LABORATORY could not conduct its research work without the assistance of the CellSearch® platform – only five other labs in the country have access to this technology. This system is currently the only CTC platform that has been cleared by the US Food and Drug Administration and Health Canada – but how does it work?

The CellSearch® system detects CTCs based on their protein expression. Firstly, it selects cells positive for the epithelial cell adhesion molecule EpCAM – which is expressed exclusively in epithelia and epithelial neoplasms. Then, using antibody-based differential staining, cytokeratins eight, 18 and 19 are flagged, as well as CD45, a protein expressed by white blood cells. Finally, a nuclear stain is performed to highlight cells with a nucleus.

When CellSearch® is applied to a blood sample from a patient, the analyser scans the sample and takes images of these stains. In order to be considered a CTC, a cell must be positive for EpCAM and cytokeratin, negative for CD45, contain a nucleus, and be at least four μm in diameter. When a 7.5ml sample of blood contains five or more CTCs – in prostate cancer cases – it is associated with markedly reduced survival.