Seeds4Hope

A program of the Windsor Essex County Cancer Centre Foundation

2012 Seeds4Hope Grant Recipient - *Dr. Michael Boffa*

"Is Thrombin Activatable Fibrinolysis Inhibitor (TAFI) a Novel Anti-Metastatic Factor in Breast Cancer?"

SUMMARY OF RESEARCH PROJECT

Most cancer deaths occur not from the original tumour, but as a result of spreading of tumour cells to distant locations in the body, a process known as metastasis. In order for tumour cells to spread, or metastasize, they need to acquire certain characteristics and also require assistance from non-tumour cells nearby. First, the tumour cells needs to break free of the main tumour and migrate through the surrounding tissue. Then, the cells must enter either blood or lymphatic vessels which allows them to travel to distant organs. Finally, the tumour cells must be able to invade the distant organs and then continue to grow in their new environment.

Many of these processes are dependent on the ability of tumour cells to make or to control enzymes, called proteases, which digest proteins. In doing so, tumour cells can digest their link to other cells, can digest tracks for themselves in the protein matrix which surrounds them, and can penetrate blood or lymph vessels. In our proposal, we are interested in a particular protein that appears to control how much protease activity is developed by tumour cells. Therefore, we hypothesize that this protein, called thrombin activatable fibrinolysis inhibitor (TAFI), might prevent metastasis by decreasing the protease activity around tumour cells. TAFI was originally discovered on the basis of its ability to regulate the breakdown of blood clots. However, more recent research from our laboratory and others suggests that TAFI might be present in the tumour microenvironment and might regulate protease activity.

In order to address our hypothesis, we will undertake experiments in cancer cells grown outside the body as well as in mice. We will use sophisticated 3-D microscopy techniques to measure the ability of TAFI to regulate the protease activity of several different breast cancer cell lines. We will also measure the effect of TAFI on the ability of these cells to move in a gelatin-like matrix that simulates the environment in which they would move in the human body. If we stimulate TAFI activity, we expect that the cells will show decreased protease activity and movement, which would be consistent with our hypothesis that activation of TAFI could represent a future treatment strategy to prevent metastasis. We will also measure the effect of TAFI on the ability of cancer cells to cause new blood vessels to grow, a process known as angiogenesis. We expect that TAFI will reduce angiogenesis, and thus make it harder for tumour cells to escape into the circulation.

Finally, we will evaluate if stimulating TAFI activation can reduce metastasis of breast cancer cells in a mouse model. We will engineer cancer cells to express various mutant forms of a protein called thrombomodulin that either do or do not specifically stimulate activation of TAFI. We will then introduce the breast cancer cells into mice; since we believe that TAFI helps prevent metastasis, we expect to find that cells expressing thrombomodulin capable of supporting TAFI activation will exhibit a lesser extent of metastasis. We expect that our research will be the starting point for the development of new drugs that activate TAFI in the vicinity of tumours, thus preventing them from metastasizing.

HOW THIS RESEARCH HELPS ADVANCE QUALITY CANCER CARE IN OUR COMMUNITY

Although existing anti-cancer drugs and radiation are effective at stopping the growth of cancer cells, there is an urgent need to develop new therapies that prevent cancer cells from spreading. All new drug discoveries start with basic research, like we have proposed, to find out how cancer works and to test new ideas for anti-cancer therapies in the laboratory. One day, we hope that our discoveries will be translated into new anti-cancer therapies. Moreover, our collaborative research venture will generally strengthen the cancer research community here in Windsor and will help to bring together basic researchers and clinicians to tackle cancer research problems.

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PROGRESS REPORT - YEAR ONE

We have made significant progress on the original objectives of this project. We have completed analysis of a specific thrombin activatable fibrinolysis inhibitor (TAFI) known as PTCI, to enhance migration and invasion of two different types of breast cancer cell lines (SUM149 inflammatory breast cancer and MDA-MB-231 triple negative breast cancer). We have also demonstrated that enhancement of TAFI activation by the addition of thrombomodulin has the opposite effects: inhibition of migration and invasion. These latter findings were crucial because it is our hypothesis that stimulation of TAFI activation could be a novel strategy to prevent breast cancer metastasis. We have also completed analysis of the ability of PTCI to induce extracellular proteolysis using a fluorescent marker of collagenase activity in both breast cancer cell lines. **In all cases, the data are publication-ready in terms of the number of independent experiments performed, quantitative analysis of the data, and the robustness of the statistical analysis.**

In order to complete a manuscript including these finding, we need to employ mutant versions of thrombomodulin that are not able to support activation of either TAFI or the competing substrate from thrombin-thrombomodulin, protein C. In the past year we have made excellent progress towards this goal as well, as we have established two different expression systems for thrombomodulin (either membrane-bound like natural thrombomodulin or non-membrane bound which is a form that could be used as a drug). In addition, we have constructed all of the required mutant versions of both forms of thrombomodulin. The thrombomodulin variants are required not only for completion of the studies of breast cancer cells *in vitro*, but also for the studies to be completed next year on the effect of thrombomodulin on metastasis of human breast cancer cells in zebra fish (*in vivo*). With respect to this latter aim, in the past year **Dr. Lisa Porter and I were successful in obtaining an NSERC Research Tools and Instruments grant to purchase a state-of-the-art apparatus for executing the zebra fish experiments.** This apparatus will be received in the very near future and we will initiate our in vivo experiments immediately thereafter.

We have completed analysis of thrombomodulin and TAFI expression in a variety of breast cancer cell lines that vary in their malignancy. Expression of thrombomodulin is inversely related to malignancy (which mirrors what has been observed in human breast cancer specimens) while there is no relationship between TAFI expression and malignancy. However, more malignant cells would be less able to support TAFI activation because of their decrease in thrombomodulin expression.

As part of our move into zebra fish as a model system, we have begun to characterize the zebra fish form of the protein and to compare its properties to human TAFI. This is crucial as human thrombomodulin will be used to activate zebra fish TAFI in vivo. **We have prepared a system to produce zebra fish TAFI for analysis, and are on the cusp of obtaining sufficient material for these experiments.**

Finally, we have embarked on a separate aim presented in our original application, examining the role of TAFI in the ability of breast cancer cells to stimulate angiogenesis, the growth of new blood vessels to nourish the tumour and provide an escape route for metastatic cells. We are in the process of establishing culture conditions wherein we grow cancer cells and endothelial cells (which can form blood vessels spontaneously) together. **We have tested several methods to mark the cells with differently-coloured fluorescent markers so the properties of both cell types can be independently measured in the same sample, and have very recently hit on the appropriate combination of markers.**

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PROGRESS REPORT - YEAR TWO

Tumour metastasis is responsible for the majority of cancer related deaths. It is therefore imperative to develop methods to prevent primary tumours from spreading to distant parts of the body. Proteins in the tumour micro-environment play a vital role in tumour metastasis. My research is aimed at determining the effect of a clot-stabilizing protein, thrombin activatable fibrinolysis inhibitor (TAFI), on breast cancer invasion and metastasis. We have shown that inhibition of the active form of TAFI increases breast cancer cell invasion and migration. Moreover, we have found that inhibition of TAFI promotes the formation of new blood vessels associated with cancer cells, a process known as angiogenesis. These findings indicate that TAFI plays an important role in the tumour microenvironment, and that therapies designed to stimulate TAFI activation in this environment may constitute a novel strategy to prevent metastasis. We would like to further assess the effects of TAFI on breast cancer using animal models. The zebrafish model has proven to be an effective, affordable and practical model to study several aspects of cancer, including metastasis. In order to evaluate the effect of TAFI on breast cancer metastasis using the zebrafish model, breast cancer cells will be injected into zebrafish embryos and the migration of the cells will be tracked using a microscope; activation of TAFI will be manipulated in this model to assess the effects of TAFI on metastasis. In addition, the effects of TAFI on tumour metastasis will also be studied in mice using well-characterized approaches. Together these studies will be important in determining the functional role of TAFI in the tumour microenvironment with the use of a whole organism. Targeting TAFI may prove to be a beneficial therapeutic approach to prevent tumour invasion and metastasis.