Seeds4Hope

A program of the Windsor Essex County Cancer Centre Foundation

2012 Seeds4Hope Grant Recipient

Dr. Elizabeth Fidalgo da Silva

"The Role of Tuberin in the Development and Progression of Pediatric Brain Tumours"

SUMMARY OF RESEARCH PROJECT

Despite improvement in treatments, brain cancer remains the second leading cause of cancer-related death in children under 19 years of age and the third in young adults ages 20-39 years. Medulloblastoma (MB) is the most prevalent of all childhood brain cancers, with average 5-year survival rates of ~62% for children aged 0-14 years. To improve these statistics and quality of life after the disease, we need more effective methods to guide treatment decisions and more directed therapies.

One of the leading priorities of Cancer Care Ontario is to lessen the risk of Ontarians for developing cancer. We expect that the proposed work will reveal the role that the tumour supressor protein Tuberin plays in suppressing MB. Identifying the role of this important protein in different types of MB is critical because it will aid in properly directing therapies for patients in the future. This means that patients diagnosed with MB will have a better quality of life during and after treatment, and that fewer people will die from this form of cancer.

HOW THIS RESEARCH HELPS ADVANCE QUALITY CANCER CARE IN OUR COMMUNITY

This project holds tremendous promise for the development of novel therapeutic strategies, as well as potentially providing prognostic tools that could aid in guiding treatment decisions, for the most prevalent form of brain cancer to affect children, medulloblastoma. Such advancement would have a tremendous impact on survival rates, thereby having high priority for advancing cancer care both locally, regionally and beyond. In addition, this work will promote collaborative research with the Windsor Regional Cancer Program and other centres with expertise in brain cancers such as Sick Kids Hospital in Toronto and McMaster University in Hamilton. Strengthening ties between our local university and hospital with other medical centres in the province is very important for training, recruitment and retention of medical experts. Strengthening these ties can also attract other funds necessary to expand the innovative technologies that are developing in the Windsor area and which are essential for keeping Windsor cancer care at the forefront in this country.

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PROGRESS REPORT - YEAR ONE

Medulloblastomas (MB) are the most common and malignant childhood brain cancers. They are invasive and rapidly growing. Statistical data show an average 5 year survival rate for children with this disease. Current treatments pose long-term side effects and one-third of the patients remain incurable after these aggressive treatment regimens. Research data support that brain tumour stem cells (BTSC) may play a key role in the initiation and progression of cancers such as MB. The balance between growth and proliferation of stem cells needs to be carefully regulated. Tumour stem cells can develop if this balance is disrupted. The tumour suppressor protein Tuberin is known to be part of pathways related to cell growth and proliferation. Very little is known about the role of Tuberin in BTSC formation. The goal of my research proposal is to understand the role of the protein Tuberin in normal brain formation (neurogenesis) and to determine whether altered expression of this protein is implicated in the development of the BTSC population.

In-Vivo **Studies:** Our in-vivo studies in Balb-C mice have demonstrated that during embryo development, the level of Tuberin expression varies in distinct regions of the brain between days 14 and 16.5, times when neurogenesis reaches a peak and continues through prenatal development. This suggests that the expression of Tuberin is tightly regulated when neurogenesis is at a peak, and therefore plays an important role in this process.

In-Vitro **Studies**: We have isolated and cultured primary cerebellar cells in Balb-C mice, and have stimulated these cells to differentiate into more specialized cells. We collected cells during several time points (0 to 24 hours) during the process of differentiation. The cells were then analyzed for the levels of Tuberin and several stem cell fate and proliferation markers to ensure that the cells were indeed differentiating. We observed that the levels of Tuberin increased significantly between 0 to 16 hours and decrease from 16 to 24 hours, demonstrating that Tuberin levels are tightly regulated during differentiation, and supporting a potential role in this process. The expression profiles of the genetic markers for cell fate and proliferation suggest that the cells are differentiating away from a neuronal phenotype, and moving towards a glial cell fate.

Since MB tumours can arise from progenitor or stem cells, as per the BTSC hypothesis, we have begun to investigate the differential levels of Tuberin, and a variety of cell fate markers in the stem-like sub-population of primary cerebellar cells. To enrich for the stem-like progenitor cells within the primary cerebellar cell culture, a clonal neurosphere assay was conducted. The Balb-C primary cerebellar cells were stimulated through the use of the specialized culturing methods to form spheres of variable sizes which were sub-cultured for two more clonal passages of spheres prior to harvesting, resulting in three clonal generations of spheres. We observed a statistically significant increase in Tuberin levels over clonal regeneration of neurospheres, supporting the hypothesis that Tuberin plays an essential role in the early fate decisions of the cells constructing the nervous system. Our work over the next year will further elucidate this.

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PROGRESS REPORT - YEAR TWO

The most prevalent of all childhood brain cancers is medulloblastoma (MB) with average 5-year survival rates of ~62% for children ages 0-14 years. Research data support that brain tumour stem cells may play a key role in the initiation and progression of cancers such as MB. Tumour stem cells can develop if the balance between cell growth and cell proliferation is disrupted. The tumour suppressor protein Tuberin is known to be part of pathways related to cell growth and proliferation. My research focus on understanding the role of the Tuberin in normal brain formation (neurogenesis) and to determine whether altered expression of this protein is implicated in the development of brain tumour stem cells population.

***In-vivo* Studies:** Results of our research obtained during the first year of the Seeds4Hope grant obtained by quantifying the amount of Tuberin in Balb-C mice brain sections demonstrated that Tuberin expression is tightly regulated during neurogenesis. During the second year we increased the number of brain sections to confirm our previous results and to permit statistical analysis. The results obtained from brain sections immune-stained with antibodies against markers of different neurogenesis phases demonstrated that Tuberin expression is higher in the early stages of neurogenesis. This suggests that Tuberin has a regulatory role in neurogenesis.

***In-vitro* Studies:** Cerebellar cells were extracted from the cerebellum of Balb-C mice and cultured in cell culture dishes. These cells were cultured for neurosphere formation (stem cell proliferation) and for cell differentiation studies. We have started to manipulate Tuberin expression in these cells using the knock-down system. This system provides an excellent evaluation method, since we can identify the pathways altered in the absence of the protein of interest, clarifying its role. We have constructed four knock-down systems up to now and at the moment we are testing them. Our preliminary results show aberrant neurosphere growth in the absence of Tuberin, implying the important role of Tuberin for stem cell renewal.

During the third year of this project we will apply statistical analysis to our results obtained with brain sections. We will quantify the expression of Tuberin and neurogenesis markers in these sections and stain them with antibodies against downstream targets of Tuberin to verify which pathway/pathways (growth/proliferation) this protein is regulating during neurogenesis.

We will extract and culture brain primary cells from embryonic mice to compare with our results obtained with postnatal mice. We will work with Tuberin knock-down systems developed during the past year to clarify the role of Tuberin in stem cell renewal and differentiation.

Our studies are clarifying the role of the tumour suppressor Tuberin in controlling stem cell proliferation and differentiation during brain development. These studies hold tremendous promise for the development of novel therapeutic/biomarker strategies which may improve current brain cancer survival rates dramatically.