Seeds4Hope

A program of the Windsor Essex County Cancer Centre Foundation

2011 Seeds4Hope Grant Recipient

Dr. Otis Vacratsis

"Functional Characterization of hYVH1/DUSP12: A Putative Oncogene Overexpressed in Late Stage Cancers"

SUMMARY OF RESEARCH PROJECT

To maintain cellular balance, human cells possess sophisticated molecular circuits that monitor cell size and nutrient availability. These circuits then convey this information to master regulators which control DNA replication and cell division. The exact chain of events that achieves this sophisticated communication remains unclear. What is certain is that disruption of this system is a hallmark of most cancers which allow the tumour cells to grow and divide with no regards to nutrient needs or the integrity of the genome.

The proposed research focuses on investigating a novel cell survival enzyme (hYVH1) that has recently been implemented in both cell growth and cell division. Importantly, the hYVH1 gene has been found to be overproduced in many aggressive late stage cancers including advanced brain tumours, soft tissue tumours, leukemias, and retinal tumours. Interestingly, a general property of late stage tumour cells is their ability to divide and survive under stressful conditions including low oxygen and low nutrients. We predict that overproduction of a cell survival factor such as hYVH1 that also encourages cell growth and division, provides a "triple threat" allowing late stage tumour cells to survive and flourish in sub-optimal cellular conditions. Therefore, we aim to examine if the hYVH1 gene product represents a critical link between tumour growth and tumour survival.

HOW THIS RESEARCH HELPS ADVANCE QUALITY CANCER CARE IN OUR COMMUNITY

Results obtained from this research project would present a new window of opportunity for the treatment of some of the most aggressive and chemo-resistant leukemia by a non-toxic plant extract. Furthermore, identification and characterization of the active component of the DRE would lead to the possibility of concentrating, and thus making a better formulation, of the extract to be used for treatment. The biochemical knowledge of how this extract kills cancer cells could transform the understanding of natural compounds as chemotherapeutics and open a new window of opportunity to treat blood cancer with non toxic natural extract.

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PROGRESS REPORT

We have been investigating the functional properties of an evolutionary conserved phosphatase enzyme named hyvh1 (also known as DUSP12). The hYVH1 enzyme is a unique dual specificity phosphatase enzyme that we have recently shown to have a role in cell survival and cell cycle regulation. Others have shown that hYVH1 controls protein synthesis. Although still poorly characterized, recent genetic studies have observed the hyvh1 gene to be significantly amplified in various late stage cancers including ependymomas, soft tissue sarcomas, and retinoblastomas, highlighting the importance of examining the oncogenic potential of hYVH1. We have been also interested in examining an unpublished observation that hYVH1 expression may be elevated in adult glioblastoma. Below are some progress we have made on this topic. Now that the grant is completed, we will utilize some funds from our NSERC Discovery Grant for any future experiments, until we secure additional cancer-specific funds for advancing this project in the near future.

Expression of hYVH1 protein in advanced cancer - one of our research objectives was to investigate if protein expression of hYVH1 was elevated in the same cancers that have shown gene amplifications. To accomplish this we have been continually optimizing an in-house generated anti-hYVH1 antibody to be used to analyze the expression of hYVH1. We have established that this antibody can detect high expression of hYVH1 in various cancer cell lines such as MCF7 (breast cancer), HTC-116 (colon cancer) and U20S (osteosarcoma cell line). We also have preliminary data suggesting that U251 and U87 (both adult glioblastoma cell lines) express high level of hYVH1 while SF188 (pediatric glioblastoma cell line) do not express high levels. Over the past few months we have been also attempting to optimize our hYVH1 antibody for use in immunohistochemistry (IHC) experiments. However, we have yet to have developed an optimized procedure for our current antibody. We plan on attempting a few more conditions before concluding that our antibody will not be sufficient for IHC experiments. We are currently producing a new antibody from a different organism that we hope will be able to complement our current antibody, which is good for immunoblotting and immunoprecipitation. Development of an antibody for IHC will be needed before we can collaborate with WRH collaborators on tissue microarrays from various advanced stage cancers for expression levels of hYVH1.

Identification of the hYVH1 interactome – using a functional proteomic platform during my sabbatical at McGill University, I had preliminary evidence that hYVH1 associates with a variety of proteins that function in mRNA regulation (splicing, translation, repression). Thus, we have been working on reproducing my work at McGill and validating my findings. One of the major obstacles of reproducing my McGill work was the lack of proteomic mass spectrometers at the University of Windsor. However, a recent successful CFI grant will see the University of Windsor purchase a new cutting edge proteomic system that will be up and running by January 2014. Thus, we will be in excellent position to reproduce our interactome findings not only in the osteosarcoma cells originally used but also in multiple cancer cell lines of interest.

We have also begun developing a hYVH1-specific biomarker assay using quantitative multiple reaction monitoring mass spectrometry (MRM-MS). This high throughput technique involves setting the mass spectrometer to detect only specific mass values of interest in order to detect target molecules at very low levels (e.g. levels found in biopsies or blood draw). To date, we have developed a MRM scan that can detect purified hYVH1 at

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PROGRESS REPORT *cont'd*

low femptomole levels, which is within the range for many cancer biomarkers found in human specimens. With the new proteomics system set to be functioning in January 2014, we will now be able to optimize this technique further to detect hYVH1 within complex mixtures that are analogous to clinical samples. Such a method could have potential use as an inexpensive diagnostic tool for rapidly screening high numbers of clinical samples for hYVH1 expression abnormalities.

Conference Presentations:

- Dept. of Medical Genetics, University of Toronto - November 2, 2012.
- Dept. of Chemistry and Biochemistry 50 year Alumni Symposium, U. Windsor – Oct.26, 2013
- Conference on Signaling in Normal and Cancer Cells, Banff, AB – March 23, 2014

Manuscripts:

- Geng Q, Bonham CA, Xhabija B, Mailloux CM, , Vacratsis PO. (2013) Interactome analysis reveals hYVH1as a Novel Regulator of Ribonucleoprotein Particles. J. Biol. Chem., in preparation*

***NOTE:** We were going to submit this manuscript in September after some positive validation experiments. However, we have decided to wait until we reproduce our proteomic/interactome mass spectrometry findings using the new proteomic instrument set to arrive in January 2014 to be absolutely certain of our findings and to able to provide statistical rigor. This study was partially funded by our Seeds4Hope grant and our NSERC Discovery grant and will be submitted to the Journal of Biological Chemistry.

Highly Qualified Personnel Training:

I am currently funding one MSc student (Robert Gombar) using Seeds4Hope funds. However, I also recruited an excellent PhD student from China (Qiudi Geng) to work on the Seeds4Hope project. Qiudi began her PhD studies in September 2012, and was awarded the prestigious Trillium Scholarship which pays $40,000 per year for four years. Only 75 per year are awarded throughout the province of Ontario. Qiudi is reproducing the interactome findings and is performing the validation experiments demonstrating that hYVH1 associates with mRNA particles. Also, I have recruited Dr. Aaron Steevensz as a post-doctoral fellow. Dr. Steevensz also has his own fellowship and is the principle scientist working on the hYVH1 MRM biomarker project.