Seeds4Hope

A program of the Windsor Essex County Cancer Centre Foundation

2010 Seeds4Hope Grant Recipient

Dr. John Hudson

"Regulation of the expression of Plks in myelodysplastic syndromes (MDS) and hematological malignancies"

SUMMARY OF RESEARCH PROJECT

Leukemia and lymphomas can often be spurred on by abnormal gene expression including shutting down or turning on important genes. Our research is focused on the polo-like kinase (Plk) genes (Plk 1-5) which play important roles in preventing damaged DNA from being passed on to dividing cells and regulating normal cell division. One of the Plk members, Plk2 has been previously shown to be abnormally silenced in blood malignancies (B-cell lymphomas). We have found in liver, lung and other cancers, that the abnormal silencing of Plk4 and activation of Plk1 is correlated with the progression of tumours. The purpose of our research program is to better establish whether the Plks play a role in the development of various types of hematological cancers such as myelodysplatic syndromes, leukemias and lymphomas. Previous evidence suggests that the Plks may prove to be important targets for diagnosing and treating these malignancies. Therefore, once we establish the relationship between each Plk and an individual hematological disorder, we intend to use pharmacological agents to help restore the gene expression landscape, with the purpose of promoting normal gene expression and allowing cancerous cells to revert back to normal. We will be screening and treating both normal and tumour types of cells as well as biopsy samples. Recent studies indicate that the use of cholesterol lowering drugs or statins inhibits the growth and progression of prostate cancer as well as reducing breast cancer incidence by up to 18%. Despite these exciting correlations, the manner in which increased cholesterol leads to breast cancer initiation and/or progression is not well understood.

HOW THIS RESEARCH HELPS ADVANCE QUALITY CANCER CARE IN OUR COMMUNITY

All evidence to date suggests that the Plks are extremely important regulators of the cell, which, once abnormally expressed may contribute to the development of malignancy. Very little is known regarding their role in hematological malignancy. Our goal throughout this project is to advance our understanding of hematological malignancies in the context of the Plks with the hope of translating these discoveries to better patient outcomes. This project is a collaboration between my laboratory at the University of Windsor, and local oncologists and pathologists at the Windsor Regional Cancer Centre and Windsor Regional Hospitals. We will be using both cell lines and clinical samples to establish potential links between the Plks and hematological malignancy. Once these are established we will be using drugs that are already in use at the Cancer Centre or in clinical trials within North America to further characterize this relationship. This research has the potential to tell us which patients may be more susceptible to progression of disease, which patients are likely to respond to or are responding to treatment, and whether the treatment regime is advantageous or detrimental. In conclusion, our research could provide better screening methods and drugs that would prevent, diagnose and/or treat patients from our community with MDS, leukemias and lymphomas.

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PROGRESS REPORT

Lymphomas, myelodysplastic Syndromes (MDS, formerly known as pre-leukemia), and other hematological (blood related) cancers are generally associated with abnormal changes in the genes required for normal cell function. These changes can potentially disrupt the standard processes in cells which results in cells dividing in an uncontrolled fashion, a hallmark of cancer. In some cases, changes in the regulation of a gene may occur through modification of the DNA without any mutations occurring, but rather by altering the ability of the cell to use the information stored in the gene sequence. This type of regulation is known as epigenetics. The focus of our research involves studying the role of the polo-like kinases (*plks*) in malignancy. In humans, there are five members of the *plk* family (*plk1-5*) all of which have been implicated in a wide variety of cancers. Through the funding received from Seeds4Hope, we sought to determine whether the epigenetic changes within the polo like kinase (*plk*) family of genes, may play a role in the aforementioned hematological malignancies and whether this is of clinical value. One type of epigenetic modification that we have focused on is DNA methylation. For the polo-like kinase genes, increased DNA methylation of gene is typically correlated with decreased expression (or turning the gene off).

In general, abnormal regulation of the polo-like kinases (*plks*) is known to contribute to tumour development. For example, in many cancers a class of genes that encode tumour suppressors, proteins that prevent cells growing in an uncontrolled fashion, are often turned off by methylation of their DNA. *Plks2-5* are typically at lower levels in cancers and are found to play a role in the cell as aides in tumour suppression, while *plk1* is normally over-expressed in many tumour types and is associated with poor prognosis.

Recent findings from our lab have shown that there is a large proportion of malignant samples that have a profile in which the *plks* are abnormally expressed. Approximately 20% of the aberrant samples we have examined have higher levels of *plk1* compared to the normal counterparts. Conversely, between 60-80% of the samples have lower levels of *plk2-4* compared to normal bone marrow aspirates. In addition, we have also determined that the tissue environment plays an important role in the way these genes are regulated. Moreover, these patterns are detectable in peripheral blood, bone marrow aspirates, and bone marrow biopsies, suggesting that this may be a biomarker. Interestingly, we know that epigenetic modifications are reversible and there are therapeutic treatments that are in clinical use that accomplish this. Therefore, we have exposed cells derived from hematological cancers to these drugs to determine if they help to re-establish normal *plk* expression patterns. We have discovered that the effect of the treatments on *plk* expression is dependent on the oxygen levels of the growth of environment, such that differences in expression have been observed between standard oxygen (16-18%) and low levels of oxygen (5%) normally found in tissues.

We are currently in the process of completing our analyses by incorporating the clinical data associated with each sample. Our results to date suggest that *plk* gene status may be a potential diagnostic or prognostic indicator in hematological malignancies.

Conference Presentations:

- Alejandra Ward, Gayathri Sivakumar, Caroline Hamm, Sindu Kanjeekal, Andrei Cerghet, Anna Kozarova, John W. Hudson. The role of epigenome-modifying drugs on the polo-like kinase promoter methylation in hematological malignancies. Accepted. American Society for Cell Biology (ASCB) Annual Meeting New Orleans, Dec. 2013.

- Alejandra Ward, Gayathri Sivakumar, Caroline Hamm, Sindu Kanjeekal, John W. Hudson. (2102). Deregulation of promoter methylation of the polo-like kinases in myelodysplastic syndromes (MDS) and other blood neoplasms. American Association for Cancer Research (AACR) Annual Meeting Chicago April 2012. LB-376

- Gayathri Sivakumar, Alejandra Ward, Caroline Hamm, Sindu Kanjeekal, and John W. Hudson. (2102) Effects of hypomethylating agents of the methylation status of the Polo-like kinases in hematological malignancies. Windsor Cancer Research Group (WCRG). First annual conference. Windsor, ON Canada 2012

Manuscripts in Preparation:

- Alejandra Ward, Gayathri Sivakumar, Sindu Kanjeekal, Caroline Hamm, Brayden Labute and John W Hudson. Plk4 promoter methylation status as a potential biomarker in a subset of haematological malignancies (2013)

Highly Qualified Personnel Training:

Funds from the Seeds 4 Hope grant contributed to the training of three graduate students and one continuing student (will be a graduate student in Jan 2014) during the tenure of the grant. Alejandra Ward PhD candidate, Gayathri Sivakumar, Brayden Labute, MSc candidates and Andrei Cerghet (continuing student). Both Ms. Ward and Sivakumar are scheduled to graduate by Feb 2014.

Awards:

My graduate students Alejandra Ward and Gayathri Sivakumar were awarded the Life Technologies best poster prize at the American Association for Cancer Research (AACR) Annual Meeting in Chicago in April 2012.