

## RESEARCH REPORT

Name: Timothy J. Storr	Affiliation (University): The University of British Columbia	
Research Advisor: Prof. Shigenobu Yano	Host Institution: Nara Women's University	
Research Subject: Synthesis of novel rhenium and technetium complexes containing pendant carbohydrates for radiopharmaceutical applications		

### **1 . Research Description:**

The goal of this research project is to synthesize novel rhenium and technetium complexes containing carbohydrates for radiodiagnostic and radiotherapeutic applications. Making use of transport and metabolic pathways for carbohydrates in the body is an attractive method of targeting radiopharmaceuticals. Currently, 2-[<sup>18</sup>F]-fluoro-2-deoxy-D-glucose (FDG) is the pre-eminent radiotracer used for localization of tumor and metastatic tissue as well as imaging brain abnormalities. Due to the production costs and short half-life of <sup>18</sup>F the utility of FDG is limited to only a few hospitals and research centers. For this reason we endeavored to produce analogs of FDG containing <sup>99m</sup>Tc and <sup>188</sup>Re. <sup>99m</sup>Tc is the most commonly used radioisotope in nuclear medicine and is readily available in most hospitals. <sup>188</sup>Re is also easily obtained and has the potential to be used in radiotherapeutic applications.

### **2 . Research Activities:**

My research activities in Japan centered on the synthesis of rhenium complexes with sugar-pendant diamine ligands. Prof. Shigenobu Yano is an expert in metal-carbohydrate interactions and has developed a series of sugar-pendant diamine ligands. I had the opportunity to use these ligands for my study while in Japan. I investigated both rhenium(V) and rhenium(I) oxidation states and was able to isolate and characterize a number of rhenium complexes with pendant carbohydrates. The instrumentation available at the university was exceptional and allowed for easy characterization of the synthesized compounds. An X-ray crystal structure of one such compound was also determined and is the first example of a group 7 metal complex containing a sugar moiety.

### **3 . Perspective of Research after this Program:**

The research conducted in Japan over the last two months is an important first step towards the goal of developing new technetium and rhenium based radiopharmaceuticals. Initial experiments with rhenium yielded promising results laying the groundwork for future studies with the radioisotopes <sup>99m</sup>Tc and <sup>188</sup>Re. Studies will continue at my home university in Canada on this interesting project ensuring that this collaboration continues. Equally important during my stay was the opportunity to meet and interact with students and researchers in Japan. I was able to share my research findings with many scientists from different fields and received important feedback. I also learned a great deal about how research is conducted in Japan. Students in the laboratory were very kind and went out of their way to ensure that I had many interesting cultural experiences.

**4 . Advisor's Remarks:**

It seems like Tim has enjoyed his two month stay in Japan. His study progressed very well and he will continue this project upon his return to Canada. He managed to complete much of the proposed research and the results are very interesting so far. Tim has made many friends in our laboratory and was a constant source of entertainment. Our collaboration will continue and I am sure that our paths will cross again very soon.

## RESEARCH REPORT

Name: <b>Cynthia Fisher</b>	Affiliation (University): <b>University of British Columbia</b>	
Research Advisor: <b>Dr. Yoshihiro Takihara</b>	Host Institution: <b>Department of Stem Cell Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University</b>	
Research Subject: <b>MSCV retroviral vector-based RNA interference of the Rae28 Polycomb Group gene in tissue culture cells and hematopoietic cells</b>		

### **1 . Research Description:**

The ability to specifically inactivate single or multiple genes within an organism or cell culture system is a powerful tool in dissecting gene function. Within the field of mouse genetics, specific gene inactivation is accomplished through the generation of gene knockout mice utilizing the phenomenon of homologous recombination in embryonic stem cell lines. However, gene knockout studies have certain drawbacks: 1) they are very time-consuming and expensive, in part due to the large number of breeding crosses involved; 2) knockout of one gene can be compensated for by upregulation of homologous genes during development thereby obscuring the phenotype, and, in general, multiple genes can be inactivated only through interbreeding of single gene knockout mice, which is also a laborious process; 3) the gene knockout via homologous recombination method cannot be readily used to inactivate genes in pre-existing mouse or human somatic cell lines or primary cell samples; and 4) early lethality of a knockout mouse model could prevent facile investigation of functions later in development or in the adult. Within the past few years, a powerful tool called RNA interference (RNAi) has emerged which permits specific gene silencing in evolutionarily diverse eukaryotic organisms including fungi, nematodes, *Drosophila*, plants, mice, and also in mammalian embryonic and somatic cell lines. The new RNAi approaches offer several advantages compared to gene knockouts and open up many new possibilities for studying mammalian gene function. With RNAi, it is possible to downregulate specific genes in somatic mammalian cell lines and primary cells; such cells modified ex vivo can then be studied using in vitro assays or implanted into mice for in vivo assays. Development of retroviral based RNAi vectors would also be of use to apply the RNAi technique to certain mammalian cell types for which it is generally difficult to achieve stable, high-level gene expression, notably hematopoietic cells, embryonic stem cells, and embryonal carcinoma cells.

The Polycomb Group gene Rae28 has been implicated in several aspects of embryonic mouse development, most notably in heart formation and axial skeletal patterning. Apart from its role in development, Rae28 is also involved in regulating hematopoiesis, and is required to maintain the normal number of hematopoietic stem cells (HSCs). HSCs generate the entire complement of differentiated blood cells within the animal, and function throughout adult life. Knockout Rae28 mice die around birth, making it impossible to study the role of Rae28 in adult hematopoiesis. Additionally, homologous genes to Rae28 may partially compensate for loss of Rae28 function in the knockout model system during development. In order to further investigate both the function and the mechanism of action of Rae28 as a regulator of HSCs and hematopoietic differentiation, I set out to apply the RNAi technique to inactivate Rae28 in tissue culture cells. Upon successful inactivation of Rae28 protein in tissue culture cells, the technique may be applied to primary hematopoietic cells including HSCs. By eliminating Rae28 protein expression in HSCs, we can use various *in vitro* and *in vivo* techniques to study Rae28 function in regulating stem cell differentiation in the adult, and the role it plays in blood cell diseases.

### **2 . Research Activities:**

Using four different RNAi hairpin oligomers that were designed in my PhD laboratory and cloned into a traditional mammalian expression vector before coming to Japan, the initial task in Japan was to subclone these fragments, containing the U6 RNA polymerase III promoter, into the appropriate retroviral expression vector, MSCVpuro. We were successful in subcloning two of the four constructs, and so proceeded with testing the efficacy of Rae28 downregulation of those two constructs in the 3T3 mouse fibroblast tissue culture cell line. For as yet unknown reasons, it is usually necessary to pre-screen several different RNAi hairpin constructs for a given target gene in order to find one that actually works to downregulate the target gene. This task is an empirical one; however as the technology matures no doubt the process will be understood in more depth.

We chose to screen RNAi constructs for downregulation of the Rae28 gene using 3T3 tissue culture cells

as they are much easier to maintain in culture than primary bone marrow cells. However, since this RNAi technique will ultimately be applied to primary hematopoietic cells, the use of a retroviral vector is necessary to deliver the RNAi constructs, since hematopoietic stem cells and primary bone marrow cells are not amenable to more traditional means of introducing foreign DNA into cells.

We transfected purified DNA containing the RNAi constructs within the retroviral vector into PLAT-E viral producer cells, and then harvested supernatant from the PLAT-E cell culture in order to infect the 3T3 cells with the retrovirus. After one week of selection in puromycin to isolate and expand cells which contained retroviral integrations, we harvested total cell protein extracts from the 3T3 cells and assessed the protein expression levels of Rae28 within each sample by Western blot analysis. There appeared to be at least a two-fold reduction in the expression of Rae28 within each of the two test samples, indicating that the technique was working to a limited degree. However, our goal of abolishing Rae28 protein expression completely was not met; therefore additional RNAi constructs will have to be screened in the future.

### **3 . Perspective of Research after this Program:**

Conducting research in Japan has been an interesting, stimulating, and at times challenging, experience. My visit here stems from an ongoing collaboration between my PhD thesis supervisor's laboratory (Dr. Hugh Brock, UBC) and Dr. Takihara, which has been very fruitful in advancing the related research projects being undertaken in both labs as we each work on Polycomb Group genes. Fortunately all members of Dr. Takihara's lab spoke English, so communication problems were minimized since I do not speak Japanese very well. While my research in Japan is not directly applicable to my PhD research in Canada (which has almost concluded), I have gained valuable experience in Japan nonetheless. I have improved my communication skills and flexibility of working in different research environments. I was most impressed by the work ethic of researchers in Japan, and the level of collaboration that appears common among researchers within the same Departmental laboratory. I received much assistance and helpful advice from the members of the lab, without which I could not have conducted this project. However, I think the level of collaboration between different laboratories within the same University could be improved: for example, there did not seem to be much sharing of equipment between laboratories, which ultimately reduces efficiency of the research enterprise as a whole.

I was very pleased to present an official seminar on my PhD thesis work to the Research Institute, and received several insightful questions and comments from the audience that will assist me in writing my PhD thesis. I had several interesting conversations with Dr. Takihara and members of his lab, and contributed to the discussions at our weekly lab meeting sessions. Finally, on a more personal note, I have gained further understanding and appreciation of Japanese society and culture, and have made many new colleagues and friends. I was honored to attend the memorial ceremony on August 6<sup>th</sup> at the Peace Park in Hiroshima, and to participate in the lantern floating ceremony to celebrate hope for peace. I enjoyed my time in Hiroshima greatly, and look forward to returning to Japan in the future.

### **4 . Advisor's Remarks:**

Ms. Cynthia Fisher stayed in my laboratory for about 6 weeks under the Summer Program for stay in Japan. She attempted to establish a RNAi system to knockdown expression of the gene regulating chromatin structure. I believe that her challenge would facilitate her future project. And furthermore I am sure that she not only enjoyed herself in her life in Japan as well as learned Japanese culture and pacifism, a message from Hiroshima.

## RESEARCH REPORT

Name: Jenny Ellis	Affiliation (University): University of Cincinnati	
Research Advisor:	Professor Masako Kato	Host Institution: Nara Women's University
Research Subject: Vapochromic Compounds as Chemical Sensors		

### **1 . Research Description:**

The development of methods for gas and vapor sensing is critical to applications ranging from workplace safety to chemical weapons detection. One approach, which relies on the spectroscopic response of a host material to a sorbed analyte, has rekindled interest in compounds comprised of stacked square planar d<sup>8</sup> metal complexes in which the metal centers are separated by relatively short distances (<3.5 Å). Mann and coworkers have found that intensely colored double salts composed of platinum tetra-arylisocyanide dications and tetracyanide dianions, M(CN)<sub>4</sub><sup>2-</sup> (M=Pd or Pt), undergo rapid and reversible colour changes in the presence of solvent vapors. The square planar complexes stack to form infinite chains with short metal...metal interactions that are responsible for the intense colors. Though detailed structural information is not available for those systems, the “vapochromic” behavior is believed to be related to three factors: specific interactions between the analyte and the coordinated cyanide groups, changes in dielectric constant near the chromophore, and changes in the Pt...Pt separations. Recently, Masako Kato has reported a new dinuclear platinum(II) complex, syn-[Pt<sub>2</sub>(bpy)<sub>2</sub>(pyt)<sub>2</sub>][(PF<sub>6</sub>)<sub>2</sub>] (bpy = 2,2'-bipyridine, pyt = pyridine-2-thiolate ion). In the presence of organic vapors acetonitrile or ethanol the complex exhibits a remarkable change in its luminescence. However, the exact mechanism of vapochromic response is not known. This lack of understanding poses a fundamental problem, because it prevents us from rationally tailoring the response time, selectivity and sensitivity of these materials as required for chemical sensing applications.

### **2 . Research Activities:**

Comparison of emission, absorption and reflectance data for vapochromic compounds is essential for the elucidation of the relationship between electronic structure and molecular structure in the vapochromic response. During my short stay at Nara Women's University I was able to synthesize and characterize four new compounds [Pt<sub>2</sub>(4,4'-dmbpy)<sub>2</sub>(pyt)<sub>2</sub>][(PF<sub>6</sub>)<sub>2</sub>], [Pt<sub>2</sub>(5,5'-dmbpy)<sub>2</sub>(pyt)<sub>2</sub>][(PF<sub>6</sub>)<sub>2</sub>], [Pt<sub>2</sub>(ph-bpy)<sub>2</sub>(pyt)][(PF<sub>6</sub>)<sub>2</sub>], and [Pt<sub>2</sub>(ph-bpy)<sub>2</sub>(2-qlt)][(PF<sub>6</sub>)<sub>2</sub>]. It was extremely lucky that NWU has a X-ray diffractometer and Masako Kato is a crystallographer. She was able to collect X-ray crystallography data on some of the crystals which were isolated. Emission properties of the Pt(II) compounds were also collected.

### **3 . Perspective of Research after this Program:**

The *long-range goal* of this research is to fully probe the scope of this behavior and understand the relationship between molecular structure and spectroscopy in these systems. The overall objective of the research is to elucidate the mechanism of vapochromic response. This program has allowed me to develop a collaboration between Dr. Connick's lab at the University of Cincinnati and Masako Kato's lab at the Nara Women's University.

Masako Kato and her students were always very patient with me, stopped whatever they were doing to help, answered all my questions in Japanese then translated into English, and taught me a great deal about their field. I am certainly more knowledgeable in the field of vapochromism than before I came to NWU. I also feel like I became a valuable part of their team (if only for 7 weeks) because I helped the students with English presentations of their research and their communication skills in English improved greatly over the summer.

Much thanks to NSERC and JSPS for allowing me this opportunity to come to Japan.

**4 . Advisor's Remarks:**

This program was a great opportunity for both our research group and Jenny. I am sure Jenny enjoyed a lot of her experiences in the chemical research and cultural exchanges. Also, Jenny's activity stimulated all members in our group. I believe that this was a good start for our research collaboration overseas. I hope that Jenny makes much progress in her study towards her ph.D.

## RESEARCH REPORT

Name: <b>Dalibor Breznan</b>	Affiliation (University): <b>University of Ottawa / Health Canada</b>	
Research Advisor: <b>Dr. Hiroshi Mukae</b>	Host Institution: <b>Nagasaki University</b>	
Research Subject: <b>In vitro Toxicology / Biological Effects of air pollution</b>		

### **1 . Research Description:**

Epidemiological studies provide strong evidence for disease association of particulate matter (PM) air pollution with increased mortality and morbidity from respiratory and cardiovascular diseases. Previous *in vivo* studies in our laboratory, using rats, as well as a study conducted in healthy human subjects demonstrate an increase in plasma levels of endothelin in response to PM inhalation. Endothelin is the most potent vasoactive peptide, whose elevated levels are often associated with a number of cardiovascular and pulmonary conditions.

During my stay in Japan I have conducted research into the effects of various PM on the gene expression of the endothelin family of peptides in primary human pulmonary cells.

### **2 . Research Activities:**

My research goals during my stay in Japan were; to learn the techniques of primary human cell culture by culturing human bronchial epithelial cells (HBEC) and normal human lung fibroblasts (NHLF), and to learn to perform the Ribonuclease Protection Assay (RPA), a powerful molecular biology technique which can be used to simultaneously quantify several mRNA species in a single sample of total RNA.

In addition, I have actively participated in weekly laboratory meetings, as well as journal clubs.

### **3 . Perspective of Research after this Program:**

The preliminary results of my research show a differential response in the expression of Endothelin A receptor (ETAR) and Endothelin B receptor (ETBR) genes in NHLF cells, which is both, particle composition and particle concentration dependent. The samples I have generated during the course of my study at the Nagasaki University will provide materials for conducting further research into the biological effects of PM.

Conducting research in Japan was a very interesting and rewarding experience. It has provided me with an insight into the high quality Japanese research, enabled me to establish useful collaborations and provided a chance to learn new valuable techniques which I can utilize during the course of my PhD studies.

I would like to thank Prof. Kohno and Dr. Mukae, as well as Prof. Yui and Dr. Udono for providing me with the opportunity to conduct research in their laboratories, and everybody else in the laboratories for their invaluable help, as well as NSERC and JSPS / MEXT for enabling my participation in this program.

### **4 . Advisor's Remarks:**

In spite of the very short stay, it was so nice for us to have Mr. Dalibor Breznan as a visiting researcher in our laboratory under the Summer Program. Mr. Dalibor Breznan did his study very hard, and he learned biochemical techniques such as the RNase protection assay. He shared experimental techniques and idea with young graduate students here. He also had a good communication with all the people in our laboratory and got many Japanese friends. I believe that the experience and knowledge obtained in this opportunity will be useful for his research work.

## RESEARCH REPORT

Name: Cosette Choeiri	Affiliation (University):University of Ottawa	
Research Advisor: Susumu Seino	Host Institution:Chiba University	
<b>Research Subject:</b> The effect of learning on the mRNA expression of glucose transporters in the mouse brain.		

### **1 . Research Description:**

Learning and memory processing has been shown to induce a localized metabolic activation of certain brain regions, especially the hippocampus.

My research in the laboratory of Dr. Susumu Seino investigated the effect of operant conditioning on the molecular expression of glucose transporter (GLUT) 1, 3 and 4 in the hippocampus as well as the sensorimotor cortex of adult CD1 mice. Glucose transporters are responsible for the transport of glucose from the blood or from the extracellular fluid into the cells.

### **2 . Research Activities:**

Thanks to the JSPS scholarship I was able to learn various molecular techniques in the laboratory of Dr. Seino. My research project necessitated the very highly sensitive technique called 'Real time RT-PCR'. The latter is able to quantify efficiently the molecular expression (level of mRNA) of glucose transporters in brain tissue. Besides RT-PCR, I was able to learn other molecular techniques such as RNA extraction, cDNA synthesis, cloning and sequencing.

### **3 . Perspective of Research after this Program:**

My experience in the Japanese laboratory was very enriching and it greatly enlarged my knowledge as a scientist. My research project that was carried in Japan will be an integral part of my doctoral thesis. Furthermore, the new techniques I learned as well as the new collaborations that were started between my laboratory in Canada and that of Dr. Seino will help to prepare me efficiently for my career in academia.

I would like to enormously thank JSPS as well as NSERC for allowing me to have that great experience in Japan.

### **4 . Advisor's Remarks:**

I was impressed by her progress in the project in such a short time. We all enjoyed working with her. She has learned various fundamental techniques of molecular biology. We are completely satisfied with the JSPS programme.