TRAINEE SECTION

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Scientific overview: CSCI-CITAC annual general meeting and young investigator's forum 2010

Abstract

In 2010, the annual general meeting of the Clinical Investigator Trainee Association of Canada – Association des cliniciens-chercheurs en formation du Canada (CITAC-ACCFC) and the Canadian Society for Clinician Investigators (CSCI) was held between September 20 and 22 in Ottawa. Several globally-renowned scientists, including this year's CSCI/Royal College Henry Friesen Award recipient, Dr. Paul Kubes, Distinguished Scientist Award recipient, Dr. Gideon Koren and Joe Doupe Young Investigator Award recipient, Dr. Torsten Neil, discussed a variety of topics relating to the role of technology in medicine.

The meeting was well attended by clinician scientists and trainees from across Canada and offered trainees mentorship and networking opportunities in addition to showcasing their research at the young investigator forum. The aim of this scientific overview is to highlight the research presented by trainees at both the oral plenary session as well as the poster presentation sessions of this meeting. Similar to last year's meeting [1], research questions being investigated by trainees covered the spectrum of medical disciplines, encompassing both basic science as well as clinical areas, and are summarized below.

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Stem Cell Research: A future for regenerative medicine

Strides in the field of stem cell research have fueled the exciting discipline of regenerative medicine. As is particularly evident from work with hematopoietic stem cells (HSCs) and retinal stem cells (RSCs), research conducted in this field shows promising therapeutic potential. Fundamentally, the demonstrable ability of a stem cell compartment to expand can be attributed to the process of stem cell self-renewal. Elucidation of the genetic mechanisms by which this process is regulated in HSCs, for example, will allow for propagation of HSCs from limited sources, such as umbilical cord blood, for the benefit of adult patients requiring HSC engraftment and expansion. One group has shown, in mice, that the *Hmga2* gene is expressed at higher levels in HSCs isolated from E14.5 fetal liver in comparison with those isolated from adult bone marrow. This decrease in expression was found to correlate with a developmental increase in let-7b micro-RNA expression, thus revealing an important genetic regulatory pathway for HSC *in vivo* repopulating activity. Another group presented their pre-clinical study for the treatment of retinal degenerative diseases in mice, using adult RSCs. In developing a system for subretinal delivery of RSCs, researchers found a blend of 0.5/0.5 wt% hyaluronan/methylcellulose to be most effective for contiguous distribution of RSCs over large areas of the retina and for avoidance of patch-like integration and cellular aggregation.

Examining genetic aberrations and genomic instability

Genetic alterations can produce profound effects, and act as the main drivers in many diseases. If these genetic aberrations occur early, they can be responsible for a wide range of genetic syndromes, such as micro deletion syndromes. If they occur later, they can lead to genetic instability and carcinogenesis. One group studied the 22q11.2 deletion syndrome, the most common micro deletion syndrome, which can result in learning deficits, congenital heart disease (CHD) and schizophrenia (SZ). Using the Vineland Adaptive Behaviour Scale, multivari-

ate regression analyses with covariates of age, sex, CHD status, SZ status and IQ, and surveys concerning medical and social service, researchers found that adults with this micro deletion syndrome had considerable functional impairment, and this impairment was seen to a greater extent in subjects with SZ, though daily living skills were a relative strength. A different group studied genomic instability, specifically that caused by cyclin-dependent kinase 5 (Cdk5), a serine-threonine kinase that regulates ATM and p53 activity. Using mouse embryonic fibroblasts from wild type and Cdk5-null mice, they found that the absence of Cdk5 led to increased DNA damage as observed using single cell gel electrophoresis. These cells also had a higher percentage of mitosis-arrested cells and aneuploidy, demonstrating the role of Cdk5 in maintaining genomic stability and further suggesting a role for Cdk5 in carcinogenesis.

Hematologic Malignancies: Innovative approaches to treatment

There have been great chemotherapeutic advances for the treatment of hematologic malignancies yet further improvements to these regimens is required, particularly for specific types and subtypes of leukemias, to increase the rate of remission and maintenance for leukemia patients. A unique approach presented by one group uses oncolytic viruses (OVs) to infect and kill cells in a murine leukemia cell line, L1210. Infecting this cell line with an engineered OV (d51-VSV) *ex vivo*, followed by tail vein vaccination of naïve or cyclophosphamide-treated DBA mice and subsequent lethal leukemic challenge, revealed that prior chemotherapy drastically impairs OV-induced anti-leukemic immunity in comparison with non-chemotherapy-treated mice. Another group studied the anti-leukemic action of an anti-parasitic agent, ivermectin, on primary acute myelogenous leukemia (AML) samples from human patients. Ivermectin was found to preferentially induce cell death in leukemic cells, in comparison with normal hematopoietic cells, through induction of chloride-dependent cell hyperpolarization, leading to a consequential increase in levels of reactive oxygen species. Pre-clinically, ivermectin demonstrates effective treatment of AML, and is synergistically enhanced, in combination with other drugs such as cytarabine and daunorubicin. Interested in a different type of leukemia, chronic lymphocytic leukemia (CLL), another group was involved in a phase II clinical trial, initiated at Cancer Care Manitoba, to test and probe the mechanism of anti-leukemic action for valproic acid in CLL patients. Although valproic acid induces apoptosis in CLL cells *in vitro* through hyperacetylation of histones and up-regulation of death receptor 4 to further activate the TNF-related apoptotic ligand apoptotic

pathway, the clinical study revealed that valproic acid monotherapy is ineffective, but as an adjuvant to nucleoside analogue-based treatment, valproic acid sensitizes CLL cells for nucleoside analogue-induced apoptosis.

Focused more on how to improve care for patients with hematologic malignancies in medical oncology divisions, a different group developed a model to predict the length of hospital stay for these patients. This model takes into account factors such as age, age squared, port of entry to the hospital, county of residence, gender, weekday versus weekend day of admission, diagnosis and number of previous hospital admissions. Anticipation of patients at risk for prolonged length of stay would caution clinicians to consider earlier intervention.

Oncology

Cancer and tumorigenesis featured prominently in studies at the Young Investigator Forum this year. A variety of novel technologies evaluating a spectrum of tumor types were on display. The role of viruses, both in oncogenesis and as part of the therapeutic arsenal against cancers, was explored. One study evaluated the role of polyomavirus in the pathogenesis of non-melanoma skin cancer (NMSC) in both immunocompetent and immunosuppressed organ transplant recipients. Utilizing a monoclonal antibody to simian virus type SV40 large T antigen, the presence of polyomaviruses was identified in paraffin-embedded skin sections from NMSC specimens. SV40 expression was noted to be high in both basal cell carcinomas as well as squamous cell carcinomas from immunocompetent and transplant recipients suggesting a strong association between polyomavirus expression and NMSC.

Breast cancer was one of the tumor types represented at the forum. To better understand the heterogeneity within breast tumors, one study attempted to identify a method to compare and characterize the *in vivo* clonal growth properties of xenografted human mammary cells that were isolated from normal and malignant tissues. The method utilized a lentiviral vector, Lenti-MNDUS-GFP, to identify the parameters that affect the efficiency of transduction of abnormal primary human epithelial cells. The transduction noted with this vector was accompanied by evidence of significant toxicity and further optimization of the transduction protocol was felt to be warranted to allow this protocol to be used as a tracking strategy in conjunction with quantitative assays for monitoring breast cancer stem cell growth in xenografted immunodeficient mice. Another study evaluated the tumor growth potential for glycoprotein non-metastatic B (GPNMB), a transmembrane protein that is expressed in 41-74% of breast cancers. This study showed that GPNMB promotes the outgrowth of

mammary tumors *in vivo* and enhances their microvascular density. Furthermore, it was noted that the extracellular domain of GPNMB is shed by breast cancer cells and is capable of inducing endothelial migration. Researchers were also able to demonstrate that ADAM10 is a novel sheddase for GPNMB. Glioblastomas and astrocytomas were also among the tumor types that were reported on this year. The consequences of over-expression or silencing of the Y-Box-Protein-1 (YB1), a DNA/RNA-binding protein implicated in cancer progression, was studied in relation to its effects on cell proliferation and mesenchymal properties. Nuclear YB1 appears to promote cell growth while cytoplasmic YB1 appears to be important in inducing the mesenchymal phenotype - possibly resulting in growth inhibition. Furthermore, silencing YB1 resulted in increased proliferation in glioblastoma cell lines and to decreased proliferation in H-tert immortalized astrocytes. These results suggest that while targeting YB1 as a therapeutic intervention may be feasible in early astrocytomas. In later stage astrocytomas, where multiple molecular signalling pathways have been altered, targeting YB1 may result in tumor cell proliferation. Another group studied the role of defective ciliogenesis in the development of the malignant phenotype of astrocytomas/glioblastomas. Using cultured normal astrocytes, as well as five astrocytoma/glioblastoma cell lines and monospecific antibodies, this study reported novel evidence that the formation of the primary cilium was disrupted in cells derived from tumor cell lines. This finding suggests that altered primary cilia are likely part of the malignant phenotype. Prostate cancer was another tumor type that was reported on this year. A systematic review was undertaken to identify the effect of supplemental vitamins and minerals on the development of prostate cancer. Fourteen relevant articles were identified after a search of the PubMed, Embase and Cochrane databases. Two authors reviewed each of the articles using the US Preventative Services Task Force Quality Rating Criteria. Neither the primary results or sensitivity analyses utilizing higher quality studies and randomized controlled trials showed a significant association between multivitamin or individual vitamin/mineral supplementation and the risk of development of prostate cancer or death due to prostate cancer.

Molecular pathways that drive the malignant phenotype are increasingly utilized as targets for therapeutic intervention. Akt is a serine/threonine kinase that promotes survival and has been implicated as a target of phosphatidylinositol 3-kinase (PI3K) signalling in promoting malignant transformation. A study, utilizing the germ line of the roundworm *Caenorhabditis elegans* as a model for DNA damage-induced cell death, found that some PI3K components do not function upstream of the

AKT-1/akt; instead, they signal independently of AKT-1/akt to promote, and not inhibit, apoptosis. Conservation of this pathway might allow for the design of therapeutic interventions against Akt-dependant tumors. Another study investigated the relationship between surgery and the increased development of metastases noted in animal models. Surgical stress was induced in BalbC mice following tail vein injection of colon cancer cells with and without perioperative anticoagulation with subcutaneous tinzaparin. Surgery resulted in a significant increase in metastases while anticoagulation with low molecular weight heparin (LMWH) abrogated this increase. Fibrin and platelet clots were associated with tumor cell emboli (TCE) more frequently in mice that underwent surgery in comparison with mice with no surgery or those that underwent pre-treatment with LMWH. Significant differences in metastatic foci were seen at twelve hours and three days following surgery but not at earlier time points. These findings corroborate the hypothesis that the hypercoagulable state of surgery promotes formation of microthrombi around TCE in the microvasculature and that these microthrombi inhibit the natural killer cell-mediated destruction of these tumor cells, promoting the post-operative development of metastases.

Infectious diseases and inflammation

This year, the field of infectious diseases was rich in studies aimed at improving our understanding of the molecular pathways involved in inflammation and infection. One study focused on the process of necrotic cell death as a potent sterile inflammatory stimulus for chemotactic migration of neutrophils. Using a dynamic *in vivo* imaging technique, the authors identified many Danger-associated molecular patterns (DAMPs), building up a multi-step hierarchy of directional cues that initiate neutrophil recruitment to the site of inflammation. Another group investigated the role of the Carcinoembryonic Antigen-related Cellular Adhesion Molecule-1 (CEACAM1), expressed on the surface of epithelial cells, in the pathogenesis of *Neisseria gonorrhoeae* infection. The results supported a CEACAM1-dependent regulation of phosphoinositide pathways via recruitment of SHIP-2 in response to infection by *N. gonorrhoeae*, thereby contributing to neisserial inhibition of epithelial cell immune responses. A third study explored the regulatory factors in BK virus replication, the causative agent of polyomavirus-associated nephropathy (PVAN), following renal allograft transplantation. Researchers found that MAP kinase signal activation increases BK polyomavirus replication and facilitates viral propagation *in vitro*, shedding light on the mechanisms of BK virus replication in the pathogenesis of PVAN. Another group studied the dys-

regulated host pro-inflammatory responses during malaria infection by quantifying monocytes membrane and plasma soluble levels of the Triggering receptor that is expressed on myeloid cells-1 (TREM-1). They showed that malarial infection of red blood cells results in decreased surface levels of TREM-1 on monocytes and induces the release of soluble TREM-1. They also found that high plasma soluble TREM-1 levels are associated with severe malaria in pediatric patients in Uganda. Finally, the last study concerning molecular basis of infectious diseases reported the successful synthesis of two antimicrobial peptides, H2A1 and H3, which were derived from specific regions of the mammalian histone genome. The design of these antimicrobial peptides highlights a potential role for histones in the immune system, suggesting new pharmaceuticals for treating bacterial infections.

Two other studies addressing psychosocial issues of infectious disease were presented at this year's Forum. The first study explored the East Africa Maasai's perception of the link between their own health and the health of their animals, using brucellosis as a zoonotic exemplar. Although participants identified brucellosis as a threat to their health, they failed to identify it as a priority disease, in contrast to local healthcare staff and government representatives. These findings may have serious implications for future intervention design and implementation within this population. The second study addressed adherence to antiretroviral therapy (ART) in women living with HIV/AIDS in Vancouver's inner city. Using participant observation in a variety of clinical settings, the authors gained a better understanding of social and interpersonal dynamics affecting adherence to ART.

Internal Medicine

An ongoing objective of the Young Investigators Forum is to highlight the pathway of biomedical research: from basic theory to established practice. The research presented in this category provides an excellent example of a diverse array of topics encompassing everything from the basic, sub-cellular science to the clinical trial. Chronic musculoskeletal conditions were a particular topic of interest in this category and research supporting novel therapeutic targets and treatment modalities was presented. One study provided *in vivo* evidence that transforming growth factor alpha plays a role in suppressing chondrocyte metabolism in a mouse model of osteoarthritis. Another study, reported deficits in quadriceps muscle power (the product of muscle torque and velocity of contraction) in patients with knee osteoarthritis and suggested that speed-related deficits may be amenable to mechanism-specific therapy. Yet another reported on a randomized controlled trial assessing the efficacy

of scleropathy for the treatment of chronic achilles tendinopathy on self-reported satisfaction. From the results, it appears that sclerotherapy provides symptom relief to these patients, although these effects appear to decrease over the course of one year.

Novel therapies, therapeutic targets and adverse drug reactions provided the context for research presented on selected internal medicine topics. Two studies employed mouse models of inflammatory bowel disease (IBD) to test the effect of specific inflammatory mediators on histologic and macroscopic disease markers. One study used ICILIN, a chemical compound activating the temperature-sensitive ion channels that mediate local inflammatory responses. Intraperitoneal administration of ICILIN significantly reduced macroscopic damage, bowel thickness and myeloperoxidase activity, in comparison with a control group. Similarly, another study reported that administration of innate defence regulator peptides, native immunomodulatory agents that enhance bacterial clearance, markedly decreased signs of colitis in comparison with the control group.

Aside from IBD, other disease models included in this category were neonatal asphyxia, idiopathic pulmonary fibrosis and diabetes. Myocardial depression is a mortality-causing sequela of neonatal asphyxia. One study examined the effect of cyclosporine on the preservation of cardiac function in a piglet model of neonatal asphyxia and provided evidence of a dose-response, cardioprotective effect for this intervention. Another study identified two mutations in the amino-acid residues of the reverse transcriptase catalytic subunit of DNA telomerase that may be implicated in the phenotype of idiopathic pulmonary fibrosis. Finally, a large nested case-control study assessed the risk of inducing hypoglycaemia, with different diabetes medications, in patients with compromised renal function. It was observed that glyburide, which has traditionally been avoided in this patient population, does not carry an additional risk for hypoglycemic events in patients with compromised renal function in comparison with patients with normal renal function.

Diabetes is currently a major research topic, and this was reflected in this year's meeting, with two abstracts on islet cell transplantation and grafting. Establishing chimerism through bone marrow transplantation can lead to immunological tolerance in diabetes, allowing for islet transplantation. It is hypothesized that the Natural Killer (NK) cell is a barrier to induction of chimerism. One group explored the efficiency of allogeneic bone marrow engraftment and chimerism in the absence of NK cells. Preliminary data suggest that hematopoietic chimerism in mice is accomplished with greater efficiency

after depletion of recipient NK cells. Another group studied the activation of the pro-apoptotic CCAAT/enhancer-binding protein homologous protein (CHOP). Synergistic transplants were performed with CHOP-deficient or wild-type islets into diabetic mouse recipients.

Radiology

There has been recent interest in the use of imaging in cardiac diseases, and this is reflected in the posters this year. One group focused on a diagnostic application of Blood oxygen level-dependent (BOLD) cardiac MRI. In this study BOLD MRI was used to detect and quantify alterations in myocardial oxygenation in patients with coronary artery disease. Another group focused on the use of imaging intra-operatively to guide the minimally invasive delivery of a percutaneous aortic valve. In this study, information from pre-operative and intra-operative modalities was merged in an augmented fluoroscopy image.

Obs/Gynecology

There was also a focus on the impact of nutritional factors during pregnancy. Folate is vital for proper fetal development. Animal studies have suggested that alcohol impairs placental transport of folate, leading to the hypothesis that folate transfer insufficiencies may lead, in part, to the deficits observed in fetal alcohol spectrum disorders. Serum folate was measured in maternal and cord blood at delivery from alcohol-abusing mothers and controls. Results demonstrated that folic acid transport was compromised in the pregnancies affected by alcohol abuse.

The placenta is an important endocrine organ that produces hormones that can be found in both maternal and fetal circulations. It has been hypothesized that prolactin-related hormones modulate glucose levels and prevent gestational diabetes during pregnancy. One group demonstrated that prolactin gene expression is sensitive to nutrient levels in the blood and changes in response to maternal feeding.

Mechanisms of human labour remain poorly understood. Prostaglandin analogues used to induce labour interact with four different types of prostaglandin receptors. The effect of the presence of IL-1 β was studied by treating human uterine samples with pharmacological agents in the presence and absence of IL-1 β and measuring IL-8, an inflammatory chemokine upregulated with the onset of labour.

Neurological Sciences and Psychiatry

In the field of neurological science, one of the important focuses of this year's Young Investigator Forum was the cellular

mechanisms involved in the neuronal tissue response to injury. This has led to many scientific investigations aimed at further characterizing and preventing neurodegenerative disorders. For instance, one study investigated the role of p53 tumor suppressor, and its related family members p63 and p73, in the regulation of the neural precursors of the embryonic brain. The authors highlighted the importance of the balance between $\Delta Np63$ and p53 on the regulation of the apoptosis of mouse embryonic cortical precursors. Another group examined the regeneration of retinal ganglion cells following optic nerve injury by targeting the interaction between cytosolic phosphoprotein, CRMP4b, with RhoA using a competitive peptide C4RIP. In this study, expression of C4RIP in retinal ganglion cells, using an adeno-associated virus, did not promote neural regeneration of optic nerve due to insufficient protein expression; however, investigators continue to explore the potential of C4RIP to facilitate recovery after central nervous system injury. Other researchers were interested in the modulation of oxidative stress implicated in neurodegenerative diseases. The calcium-permeable non-selective ion channel, TRPM2, expressed in hippocampal pyramidal cells, is gated by ADP-ribose, ADPR, a product of oxidative stress. This study showed that TRPM2 currents increase with time *in vitro*, suggesting a susceptibility of these neurons to pathological insults in the aging brain. Similarly, using a computerized molecular dynamics simulation, another group identified regions of instability and possible subsequent misfolding in the protein superoxide dismutase 1 (SOD1), which may contribute to the pathogenesis of amyotrophic lateral sclerosis. Finally, another study aimed at enhancing our understanding of the enteric nervous system synapse regulation. Researchers investigated the role of the presynaptic cannabinoid receptor 1 (CB₁) and found that endocannabinoids acting at CB₁ receptors inhibit

neuronal transmission at enteric synapses, and these data pave the way for new therapeutic avenues for the treatment of gastrointestinal motor disorders.

In the field of psychiatry, the results of two clinical studies were presented. The first study addressed the relationship between religion and suicide. Using data drawn from Baltimore Epidemiologic Catchment Area study, the authors showed that respondents who attended religious services at least once per year had decreased risk of an incident suicide attempt, independent of the effects of comorbid mental disorders and social supports. The second study reported on the methodology of a promising mixed-methods study measuring oppression and other determinants of depression among pregnant Aboriginal women in the Calgary area. The authors of this ongoing study seek a better understanding of depression's determinants in this high-risk minority in order to develop effective interventions.

As is evident from the broad variety and the quality of research presented by MD+ trainees this year, the CSCI-CITAC annual meeting continues to provide an effective collaborative platform to further research amongst trainees across the country. We anticipate that next year's meeting will build on the research networks established amongst trainees this year and will continue to foster trainee-related research initiatives.

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References

1. Ong Tone, S., Dugani, S., Marshall, H., Shamji, M.F., Murray, J-C., and Bossé, D. 2010 Scientific overview of the CSCI-CITAC 2009 conference. *Clin Invest Med* 33: E69-72, E73-67