POSTER PRESENTATION

Poster 1
Prevalence of Body Dysmorphic Disorder in Dermatology Practices in British Columbia
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Body Dysmorphic Disorder (BDD) is defined by the DSM – IV (1994) as a preoccupation with an imagined or slight defect that leads to significant clinical distress and manifests as impairment in social, occupational or other areas of functioning. Although there is a high incidence of BDD patients presenting to dermatology offices, there has been no Canadian study on the number of patients with BDD appearing in dermatology practices. The aim of this study is to determine the prevalence of patients screening positive for BDD in dermatology offices in British Columbia. In particular, this study hopes to determine whether ethnic background has a bearing on the prevalence of screening positive for BDD. The research tool will be based on a published and validated self-reporting questionnaire, the Body Dysmorphic Disorder Questionnaire. For patients in the Chinese and South Asian community who are unable to read English, a translated questionnaire will be administered. Following the dermatologists assessment of the patient, he or she will give a defect severity score as well as a dermatological diagnosis. Once the required number of patients necessary for each group is met, the comparison of prevalence rates can be completed.

Category: Early experiments with well defined objectives/hypothesis.

Poster 2
Quantification in Dermatology with Clinical Images
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The purpose of the study will be to evaluate dermatology residents accuracy in estimating size, number, and surface area using clinical images and detailed synthetic charts. In our previous study using synthetic charts in the form of grids and pictures of household objects, participants significantly underestimated size for small objects such as coins ($p<.01$). There was a trend towards underestimation of size with large objects such as a journal; however, this was not statistically significant. Surface area was overestimated by participants ($p<.01$). Participants were most accurate at estimating number, with no bias toward underestimation or overestimation. This study will assess residents accuracy in estimating size, number, and surface area using images that will more accurately reflect the real life settings in which dermatologists estimate these parameters. In order to accomplish this, a series of 60 clinical images and 60 detailed synthetic charts will be presented in a PowerPoint presentation for 10 seconds each. The synthetic charts will have curved borders, and the areas to be estimated within them will have irregular or circular borders to represent true clinical examination situations more realistically. The clinical images will consist of ranges of size, number, and surface area of nevi on patients backs. Participants will be asked to estimate either size, number, or surface area for each of the slides. The accuracy of these estimates will be compared to true values.

Poster 3

Role of Connexins in Regulating Fibroblast Function in Wound Healing

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Objectives: Scar formation is a common unwanted outcome of wound healing. Fibroblasts are responsible for deposition of collagen-rich extracellular matrix(ECM), the hallmark of scars. Fibroblasts communicate via gap junctions (GJ) that are composed of connexins. Interestingly, blocking of connexin 43(Cx43) accelerates wound healing, but little is known about the mechanisms involved. Our first aim was to unravel howCx43 regulates expression of key genes involved in wound healing. In the second part, we compared expression of connexins in human gingival and dermal fibroblasts in association with genes regulating scar formation. Our hypothesis is that reduced expression or function of connexins in human gingival fibroblasts contributes to scar-free healing in gingiva as compared to fibroblasts from scar prone skin. **Methods:** Expression of connexins and key scar formation associated genes was analyzed using RT-qPCR, Western blotting and immunostaining. To study connexin function, we blocked Cx43 expression or function with siRNA, mimetic peptides, or chemical inhibitors. Three-dimensional *in vivo*-like cultures were used to compare the expression of connexins and above genes in gingival and skin fibroblasts. **Results:** Gingival and skin fibroblasts expressed Cx43 as their major GJ protein. Blocking ofCx43 highly regulated expression of scar formation associated genes. Compared to skin fibroblasts, gingival fibroblasts expressed significantly less Cx43, higher antifibrotic genes and lower profibrotic genes. **Conclusion:** Differences in Cx43 expression in skin and gingiva may explain the different patterns of scaring in these tissues. This information may be used to design novel therapies to alleviate tissue fibrosis, including scar formation.

Category: Early experiments with well defined objectives/hypotheses

Poster 4

RATITE OILS PROMOTE KERATINOCYTE CELL GROWTH AND INHIBIT LEUKOCYTE ACTIVATION

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Native Australian aborigines have used emu oil as a traditional wound healing treatment and anti-inflammatory remedy. Various animal studies show promising results after emu oil treatment such as promotion of reepithelialization in wound areas and anti-inflammatory characteristics. In our study, we investigated the effects of oils from emu, ostrich, rhea, duck, olive, and tea tree on immortalized human keratinocytes (HaCaT cells) *in vitro* with 0%, 0.5% and 1.0% of oil concentrations in culture medium. All of the oils were further combined with phytohemagglutinin (PHA) activated human peripheral blood mononuclear cells (PBMC) at 0.5% concentration mixture with culture medium for 48 hours incubation to evaluate their impact on the production of IFNγ with ELISpot Assays. Shorter population doubling time durations were observed for HaCaT cells cultured in emu oil (1.18X faster), ostrich oil (1.25 X faster), and rhea oil (1.14X faster) when compared to no oil control. In contrast, cells cultured with tea tree oil and olive oil showed prolonged population doubling time durations, 1.13X slower and 3.08X slower respectively. Our ELISpot results showed that individual emu, ostrich, rhea, and duck oil, exhibited a high degree of PBMC IFNγ-inhibition. This preliminary investigation suggests that emu oil has the potential to aid wound

healing by impelling the growth rate of keratinocytes and confirms its anti-inflammatory properties on human PBMC. With this combination, emu oil possesses potential in serving as a component in bandages and ointments for wound healing treatment and inflammatory skin conditions.

Category: Early experiments with well defined objectives/ hypotheses

POSTER 5

DERMATOLOGY EDUCATION IN RURAL AREAS OF BRITISH COLUMBIA: A PILOT STUDY

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In rural areas, access to dermatological care is limited and primary care needs are often met by non-physician practitioners. Remote Nursing Certified Practice (RNCP) nurses have special training that equips them for general clinical practice in remote areas where there is no resident physician. This project was designed to provide educational materials to primary providers in remote areas. The first phase involved recruiting a target group of 22 RNCP nurses. These nurses completed a pre-test dermatology quiz and survey. They were then sent a weekly series of 14 cases covering common dermatologic conditions via email. Following this first phase, a post-test and survey were distributed to evaluate the effectiveness of the program. At the beginning of our study, 18/19 nurses revealed that their comfort with diagnosing dermatologic conditions was poor or fair. Level of support by the dermatology community was rated as poor by 14/19 nurses. The estimated training in dermatology was <5 hours for the majority. Despite limited training, skin problems are a significant concern for their patients. 10/19 nurses reported that 10-25% of their patients had dermatologic problems and 4/19 reported between 25-50% of their patients had skin problems. At the end of the study, the majority felt that their ability to treat and manage skin disease had expanded and there was increased comfort diagnosing and managing dermatologic conditions. We believe this study demonstrates a clear need for increased education in dermatology for remote nurses and we propose one method for providing that education.

Category 1: Early experiments with well defined objectives/hypotheses

POSTER 6

PROGNOSTIC SIGNIFICANCE OF FBW7 IN HUMAN MELANOMA AND ITS ROLE IN CELL MIGRATION

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The E3 ubiquitin ligase Fbw7 (F-box and WD repeat domain-containing 7) is broadly considered as a tumor suppressor because of its role in turnover of several well-known onco-proteins. However, the role of Fbw7 in melanomagenes is is not clear. To investigate the expression profile and biological functions of Fbw7 in melanoma, we examined Fbw7 expression level using melanoma tissue microarray and immunohistochemistry. Our data showed that Fbw7 expression is significantly reduced in primary melanoma compared with dysplastic nevi ($P = 0.020$) and further reduced in metastatic melanoma compared with primary melanoma ($P = 0.011$). Furthermore, we observed a strong correlation between negative Fbw7 expression and a worse 5-year survival of melanoma patients ($P = 0.015$). We also found that

both Fbw7 protein and mRNA expression was significantly reduced in 9 melanoma cell lines compared with normal melanocytes. Moreover, our *in vitro* studies showed a remarkable increase of cell migration and stress fibre formation in Fbw7 knockdown cells, and treatment of selective MEK inhibitor abrogated Fbw7α knockdown induced melanoma cell migration. Taken together, our findings indicate that Fbw7 plays an important role in melanoma progression, and Fbw7 inhibits melanoma cell migration through MAPK-ERK signalling pathway and may serve as a prognostic marker.

Category: Early experiments with well defined objectives/hypotheses

POSTER 7

SCREENING FOR CYTOTOXIC T CELL ANTIGEN EPITOPE TARGETS IN ALOPECIA AREATA

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Alopecia areata (AA) is believed to be an autoimmune disease that results in non-scarring, inflammatory hair loss. Both CD4 and cytotoxic CD8 T cells (CTLs) have been found to be important for the onset and progression of AA both in humans and rodent AA models. Hair follicular (HF) keratinocyte and/or melanocyte antigens are suspected as potential targets of auto-reactive CTLs, but the specific epitopes have not yet been identified. We investigated the potential for a panel of known epitopes, expressed by human HF keratinocytes and melanocytes, to induce activation of CTLs. Peripheral blood mononuclear cells (PBMC) were isolated from AA affected and healthy subjects with HLA-A2 serotypes and cultured with synthesized HLA-A2 restrictive peptide with specific sequences for trichohyalin, melanin, MART-1, tyrosinase, tyrosinase related protein-2 (TRP2) and GP-100. The frequency of CTL activation in PBMC was measured by quantifying the number of IFNgamma secreting cells. Specific epitope cocktails derived from trichohyalin, MART-1 and TRP2 induced significantly higher responses in human AA CTLs compared to healthy controls. However, CTL activation via single trichohyalin epitopes showed highly variable results, suggesting patients with different stages of AA may have different primary targets. AA affected C3H/HeJ mouse lymph node cells (LNCs) showed significantly higher responses to mouse antigen epitopes like keratin-16 and MART-1, while trichohyalin epitopes induced minor LNC activation. The data indicate that AA affected subjects present with an increased frequency of CTLs responsive to antigen epitopes originating from keratinocytes and melanocytes. Targeting these antigen-specific cells may be effective for treating AA patients.

Category: (3)Applied/functional experiments

Poster 8

Prevention and Treatment of Inflammatory Hair Loss by Programmed Cell Death Ligand 1

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Hair loss is a common disease affecting both men and women. Although it is not fatal, patients experience extreme stress from their hair loss. Previous studies show that normal hair follicles (HF) exhibit immune privilege (IP) and loss of immune privilege may trigger inflammatory alopecia. We hypothesize that

promotion of immune privilege can prevent or treat hair loss. We identified immune privilege related genes expressed in HF mesenchyme cells by quantitative PCR (qPCR). Compared to fibroblasts, upregulation of programmed cell death 1 ligand 1 (PD-L1, a 7.3 ± 3.2 fold increase, $n = 4$, $p < 0.01$), and downregulation of MHC class I molecules (HLA-A and B showed a 2.8-, 2.0-fold decrease, respectively by q-PCR) were observed on dermal sheath cup cells (DSCs); the level of PD-L1 was increased 2.3 ± 1.2 fold tested by Western blot. Furthermore, in allogeneic responses by human peripheral blood mononuclear cells (PBMCs, as responders) co-cultured with DSCs or fibroblasts (as stimulators), the secretion of IFNg from PBMCs was significantly reduced in the presence of DSCs (23.0 vs. 5.7 pg/ml, $p<0.01$). The activation and proliferation of T-effector- ($CD8^+IFNg^+$, 32.6% vs. 26.0%, $p<0.05$; $CD8^+Ki67^+$, 12.4% vs. 4.2%, $p<0.04$) was inhibited by 20% and 66%, respectively. In addition, this hyporesponsiveness was partially removed by knockdown of PD-L1 in DSC ($CD8^+Ki67^+$, 4.2% vs. 10.4%, $p<0.05$), suggesting the requirement of PD-L1 in this inhibition. This study demonstrates that PD-L1 is a critical factor in determining IP. This study will provide a new strategy for the treatment of inflammatory alopecia.

Category (2): Early experiments with well defined objectives/hypotheses

POSTER 9

STUDY OF PAUF AS A POTENTIAL ONCOGENIC FACTOR IN MELANOMA

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Pancreatic adenocarcinoma upregulated factor (PAUF) was recently demonstrated to be a potent endothelial activator, promoting both angiogenesis and vascular permeability. Firstly, *PAUF* gene was identified to be expressed differentially in pancreatic cancer using oligonucleotide microarray. PAUF is associated with the activation of the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and AKT intracellular signaling cascades and consequently their downstream transcription factors. Also, PAUF expression is positively correlated with the activation and expression of focal adhesion kinase (FAK), a key player in tumor cell metastasis and survival. It was suggested that PAUF can upregulate and stabilize β-catenin thereby contributing to the rapid proliferation of pancreatic cancer cells. However, little is known on the tumorigenic function of PAUF in melanoma pathogenesis. We recently found that PAUF is over expressed at the transcriptional level in different melanoma cell lines compared to melanocytes. Also, we observed a strong expression of this protein in metastatic melanoma tissue samples by immunohistochemistry technique. To further investigate its role in melanoma progression, we will perform TMA analysis to investigate if increased PAUF expression is correlated with disease progression and patient survival. We will also perform in vitro assays to investigate if PAUF is involved in melanoma cell migration, invasion and angiogenesis. Our study may provide mechanistic insight into the role of PAUF in melanoma progression.

Category: Pilot/exploratory experiments (for study design, hypotheses creation, etc)

POSTER 10

JWA INHIBITS MELANOMA ANGIOGENESIS BY SUPPRESSING ILK EXPRESSION

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JWA, a novel microtubule-associated protein, is a tumor suppressor that plays important roles in apoptosis and motility of tumor cells. We previously reported that JWA knockdown in melanoma cells significantly promoted the formation of metastatic colonies. However, the potential role of JWA in tumor angiogenesis has not been elucidated, and its significance in melanoma progression is also unknown. Here, we found that JWA expression in melanoma cells significantly suppressed the tube formation of human umbilical vein endothelial cells (HUVECs). In addition, JWA regulated ILK expression through Sp1 and intergrin $\alpha V\beta 3$. Silencing transcriptional factor Sp1 abolished JWA knockdown-induced upregulation of intergrin $\alpha V\beta 3$ and ILK. We further demonstrated that JWA inhibited the tube formation of HUVECs through NF-κB/IL-6/STAT3/VEGF signaling pathway which is dependent on suppressing ILK expression. Consistently, in vivo studies also confirmed that JWA-regulated melanoma blood vessel formation was dependent on ILK expression. These findings suggest that JWA may be used as a potential anti-angiogenesis therapeutic target for melanoma.

Category: Applied/functional experiments (animal models of disease and in vivo studies, etc)

POSTER 11

DURABLE ROUGH SKIN PHANTOMS FOR OPTICAL MODELING

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Background: We previously presented fabricated skin phantoms that reliably simulated the surface roughness of human skin for laser speckle testing. Further work is needed to ensure the optical properties of the phantoms are similar to those of human skin. Objectives: To test the scattering and absorption coefficients and the anisotropy indexes of the fabricated skin phantoms. Methods: 1. Four silicone skin phantoms containing varying silica microsphere concentrations were produced to simulate the light scattering properties of skin. 2. Four pigmented silicone skin phantoms with different pigment concentrations were produced to simulate the light absorption properties of skin. 3. Actual absorption and scattering coefficients were obtained using a set of phantoms with incremental thicknesses and using the integrating spheres technique. 4. The anisotropy index for each phantom was obtained by detecting the ballistic photons passing through each phantom. Results: 1. Fabrication of skin phantoms with controllable fabrication parameters allows for control and prediction of the phantom's bulk optical properties. 2. The measured scattering coefficients and anisotropy indexes of the silica microsphere phantoms and the measured absorption coefficients of the pigmented phantoms are in agreement with reported optical properties of skin in the literature. Future Work: Fabricating phantoms with combined parameters of the microsphere and pigmented phantoms is required in order to effectively model the optical properties of skin. Clinical Significance: Phantoms simulating physical properties of skin allow us to model speckle formation for various skin conditions. This knowledge may help us develop a non-invasive and automatic device for skin disease diagnosis.

Category: pilot/exploratory experiments

Poster 12

TREATMENT OF TOXIC EPIDERMAL NECROLYSIS BY A MULTIDISCIPLINARY TEAM: A REVIEW AND DEVELOPMENT OF GUIDELINES

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Toxic epidermal necrolysis (TEN) is a rare, acute and severe mucocutaneous adverse drug reaction characterized by extensive epidermal detachment involving greater than 30% body surface area. The mortality rate has been reported to be between 25-35%. Patients with TEN are best managed in intensive care units under the care of a multidisciplinary team. Currently there is no standardized treatment protocol for patients with TEN. The aim of this study is determine the prevalence of TEN at Vancouver General Hospital and to determine factors that lead to improved survival and a decrease in associated complications. Using this information, this study hopes to establish a set of practical guidelines for the optimal management of patients with TEN, while also providing the various specialist teams involved with a unified treatment approach. The data for this study will be obtained through a thorough retrospective chart review of all patients diagnosed with TEN who were treated at Vancouver General Hospital from 2000 to 2011.

Category: Early experiments with well defined objectives/ hypotheses

POSTER 13

PROGNOSTIC SIGNIFICANCE OF THE EXPRESSION OF NUCLEAR EIF5A2 IN HUMAN MELANOMA

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The main activity of human eukaryotic translation initiation factor 5A2 (EIF5A2) was originally related to its role in cytoplasmic regulation of translation, an activity that depends on the presence of its hypusine modification. Later on, EIF5A2 was also shown to be associated with tumor progression in multiple cancer types. However, to our knowledge no one has yet shown the nuclear expression of EIF5A2. Here for the first time we are reporting the nuclear expression of EIF5A2 in human melanoma. We examined nuclear EIF5A2 expression in 459 melanocytic lesions at different stages using tissue microarray and found that positive nuclear EIF5A2 staining was significantly increased in primary melanomas compared to dysplastic nevi, and further increased in metastatic melanomas compared to primary melanomas. EIF5A2 expression was also correlated with melanoma thickness and was inversely correlated with overall and disease-specific 5-year survival of all and primary melanoma patients. Multivariate Cox regression analysis revealed that positive nuclear EIF5A2 expression is an independent prognostic factor to predict melanoma patient outcome. In conclusion, this study for the first time highlights the important role of nuclear EIF5A2 in melanoma pathogenesis and indicates that nuclear EIF5A2 may serve as a promising prognostic marker and a potential therapeutic target for melanoma pathogenesis.

Category: Early experiments with well defined objectives/hypotheses

POSTER 14

MICROPARTICULATE-ENCAPSULATED ANTIGEN WITH SPLIT TOPICAL CPG OLIGODEOXYNUCLEOTIDE ADJUVANT AS A SINGLE-INJECTION IMMUNIZATION STRATEGY

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Antigen-presenting cells (APCs) are responsible for presenting foreign antigens to, and activating T cells to fight off infection. Manipulation of APCs to modulate immune responses to immunization has been a focus in vaccinology. Keratinocytes and skin-resident plasmacytoid dendritic cells can be activated via binding of their Toll-like receptor 9 to CpG oligodeoxynucleotides (ODN) – synthetic DNA that mimic unmethylated CpG bacterial DNA. Here, we encapsulated the ovalbumin (OVA) antigen in polylactide co-glycolide microparticles (PLG-OVA) and show that mice immunized subcutaneously with PLG-OVA generate antigen-specific cytotoxic T cells when CpG ODN is used as an adjuvant. Using flow cytometric analysis, 2.3% antigen-specific cells are detected within the $CD8^+$ cytotoxic T cell population in the peripheral blood when CpG ODN is administered topically ($P < 0.05$ compared to PLG-OVA or CpG ODN alone). In addition, 0.8% of $CD8^+$ splenocytes produce interferon-gamma when re-stimulated with the OVA (SIINFEKL) peptide *in vitro* ($P < 0.001$). The microparticles show a triphasic release of OVA which simulates two doses of antigen injected separately. Using the release properties of PLG-OVA and a second topical application of adjuvant, we enhance the generation of antigen-specific cytotoxic T cells in the blood from 0.4 to 0.9% ($P < 0.05$) and OVA-specific total IgG and IgG2c production, effectively generating a single-injection vaccine with split topical adjuvant administration that induces robust cytotoxic T cell and antibody responses. Generating single-injection vaccines may enhance compliance with vaccination in the general population and may also decrease the cost of administration.

Category: Applied/functional experiments

POSTER 15

THE EXTRACELLULAR ROLE OF GRANZYME B IN DIABETIC WOUNDS OF DB/DB MICE

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Normal wound healing is a tightly regulated process involving overlapping phases of hemostasis, inflammation, granulation tissue formation and remodelling. However, in diabetics, the normal continuum of these reparative stages is often disrupted leading to the onset of chronic, non-healing wounds known as diabetic ulcers. Diabetic ulcers are characterized in part by elevated serine protease activity. Granzyme B (GzmB) is a serine protease that can mediate the cleavage and degradation of many extracellular matrix proteins that are important for normal wound healing. We hypothesize that GzmB contributes to the pathogenesis of diabetic ulcers through the cleavage of extracellular proteins. To examine the extracellular role of GzmB in chronic diabetic wounds, type 2 diabetic db/db mice will receive full thickness excisional wounds, followed by single injection of Serpina3n, a murine GzmB inhibitor, immediately after wounding. Wound closure will be monitored and quantified by planimetry to assess epithelization and contraction. Following euthanasia, skin sections will be collected to assess skin morphology and extracellular matrix alterations by immunohistochemistry. GzmB fragments will be assessed by western blot. Results from this study will provide valuable insights into diabetic ulcer pathogenesis and may identify novel therapeutic approaches for the management of chronic wounds.

Category: Pilot/exploratory experiments

POSTER 16

REPEATED CUTANEOUS INJURY INHIBITS THE DEVELOPMENT OF DIABETES IN NON-OBESE DIABETIC MICE AND INDUCES AN INFLAMMATORY PHENOTYPE IN THE SKIN

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The Non-obese diabetic (NOD) mouse is a widely studied model of spontaneous Type 1 diabetes. Interestingly, this strain is capable of producing other autoimmune phenotypes including autoimmune thyroiditis, sialitis and peripheral polyneuropathy. As cutaneous skin damage has recently been shown to precipitate persistent skin inflammation in the autoimmune prone, (NZBxNZW)F_1 mouse, we were interested in investigating whether a similar phenotype could be observed NOD mice. NOD mice and diabetes resistant NOR mice were shaved and tape stripped 10 times to induce skin damage. The inflammatory response was characterized and compared at 24 hours. Both mice developed visible lesions and a neutrophilic infiltrate. Although the lesions on NOD mice may have been more pronounced, frequencies of infiltrating neutrophils, as determined by flow cytometry were similar. To determine the effect of repeated tape injury on the development of chronic skin inflammation and modulation of diabetes, 6 week old mice were tape stripped weekly for 2 months with duct tape. At 17 weeks, none of these mice had developed diabetes (0/8 mice) in contrast to the control mice, which had a 50% incidence of diabetes (4/8 mice). Data suggests that tape stripped mice also exhibited an exacerbated inflammatory response to tape induced injury over the ear, as measured by thickness. Erythema and prolonged increases in ear thickness suggest a persistent inflammatory response. Further work in characterizing post-injury cellular events may provide insight into how cutaneous damage can modulate the organ specificity of autoimmune disease.

Category: Early experiments with well defined objectives/hypotheses

POSTER 17

COMPARISON OF SINGLE-PHOTON AND TWO-PHOTON FLUORESCENCE PROPERTIES OF HUMAN SKIN AND SKIN FLUOROPHORES

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Fluorescence spectroscopy is one of the most sensitive optical techniques for *in vivo* measurement of tissue biochemical and morphological properties. There is considerable interest in developing fluorescence-based spectroscopic and imaging techniques in biomedicine, particularly for early diagnosis of cancers and other diseases. Recently, two-photon fluorescence (TPF) imaging has gained popularity in biological and biomedical studies because of its advantages over single-photon fluorescence (SPF). Significant endogenous

fluorophores in biological tissues include collagen, keratin, elastin, nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide (FAD). Understanding the fluorescence properties, concentrations and distributions of these fluorophores within tissue is important in optimizing the spectroscopic and imaging techniques and algorithms. It is believed that the TPF properties of these endogenous fluorophores are close to their SPF counterparts. However, there is no systematic compared study on the TPF and SPF properties of these endogenous fluorophores as of to date, due partially to the limitation of instrumentation. Very recently, we developed a video-rate TPF imaging system with the capability of measuring the two-photon excitation-emission matrix (EEM) spectroscopy. In this paper, we will present the SPF and TPF properties of the above mentioned biological fluorophores and human skin using single-photon EEM and two-photon EEM systems. We find that there are similarities and differences between SPF and TPF for these fluorophores and these differences demonstrate that caution must be exercised when interpreting tissue TPF properties based on the source of SPF reference spectra or vice versa.

Category: Early experiments with well-defined objectives/hypotheses

POSTER 18

SERINE 126 PHOSPHORYLATION-DEPENDENT RECRUITMENT OF ING1B TO STALLED REPLICATION SITE

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DNA replication checkpoint plays an important role in genomic stability upon stress. However, the downstream effectors that influence such process remain elusive. We previously identified that the tumor suppressor Inhibitor of Growth 1b (ING1b) is involved in the maintenance of genome stability upon replication stress. In this study, we further showed that ING1b is recruited to the sites of replication after UV radiation. More detailed analysis revealed that ING1b is recruited to the sites of replication through a two-mode mechanism. First, ING1b is phosphorylated at S126 residue in an ATR/Chk1-dependent manner. ING1b S126 phosphorylation is required for its interaction with Rad18 and recruitment to the sites of replication. Then, ING1b further interacts with PCNA through its PIP box. Finally, we demonstrate that ING1b S126 residue is important for conferring the damage tolerance and chromosomal stability. We propose that ING1b is a downstream effector of the replication checkpoint in maintaining genome stability. Our study helps explore the mechanisms involved in maintenance of genomic stability and thereby identify the molecular targets for chemotherapeutic intervention in the treatment of cancer.

Category: Applied and Functional experiments

POSTER 19

IDENTIFICATION OF NUCLEAR LOCALIZATION SEQUENCE AND FUNCTIONAL DOMAIN OF ING3

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Inhibitor of growth family member 3 (ING3) is tumour suppressor protein which plays a crucial role in cell cycle regulation, apoptosis and transcription. We previously showed that nuclear expression of ING3 is

reduced in metastatic melanoma suggesting functional attribute of nuclear localization of ING3. However, the functional domain responsible for nuclear localization and/or tumour suppressive functions has never been addressed. We found that the nuclear localization signal (NLS) of ING3 is of classical monopartite type containing basic amino acid consensus at 164 amino acid position. Replacement of Lys164 residue of ING3 with alanine resulted in retention of ING3 in the cytoplasm. Furthermore, we examined if this NLS containing domain is essential for the tumour suppressive function of ING3 using two different mutants, ING3 K164A with point mutation and ING3 158-221 truncated mutant. ING3 WT over expression significantly inhibited migration and invasion of melanoma cells, and these effects were abrogated when K164A mutant of ING3 was expressed in the cells while the ING3 158-221 mutant had similar effect as the ING3 WT. Endothelial tube formation using the conditioned medium from cells over expressing ING3 WT, ING3 K164A and ING3 158-221 showed similar effects as in cell migration and invasion assays suggesting that 158-221 amino acid contains the functional domain of ING3. Our data suggest that over expression of nuclear ING3 may be a potential treatment strategy for melanoma.

Category: Applied and functional experiments

POSTER 20

IDO-EXPRESSING DERMAL FIBROBLASTS EXPAND A SUPPRESSIVE ANTIGEN-SPECIFIC TREG POPULATION

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Skin transplantation is often used for management of extensive burns or non-healing wounds. Although systemic immunosuppressive drugs are widely used for prevention of allorejection, side effects associated with these medications are of main concern.. Induction of immuno-tolerance, on the other hand, has several advantages to the systemic immunosuppressant, resulting in an antigen-specific tolerance of the immune system, without impairing the natural immune response to pathogens. Indoleamine 2,3-diaoxygenase (IDO) the rate-limiting enzyme in tryptophan catabolism is an immuno-modulatory enzyme, shown to increase regulatory T-cells (Tregs) population. Herein we hypothesized that $CD4^+CD25^+Foxp3^+$ Tregs, which are important in allograft tolerance, could be expanded *in vitro* by co-culturing them with IDO-expressing dermal fibroblasts (IDO-Fib). These cells could be used for induction of immuno-tolerance after allo-transplantation. To evaluate this hypothesis, murine splenocytes were co-cultured with either IDO-Fib or normal fibroblasts for 72 hours. Using FACS analysis, a higher percentage and number of Tregs were found compared that of controls. Despite observing more dead $CD4^+$ cells in the IDO group, higher numbers of live $CD4^+CD25^+$ cells were detected revealing their capacity to expand under this experimental condition . Presence of greater number of CTLA-4 $^+$ cells and high expression of TGF-β and IL-10 genes in the $CD4^+$ cells isolated from the IDO group vs. control indicated the suppressive features of expanded Tregs. Furthermore, Tregs isolated from the IDO group showed an alloantigen-specific suppressive effect in mixed lymphocyte reaction assay. These results confirm that IDO-Fibs are capable of expanding a population of suppressive antigen-specific Tregs that could be used in strategies to induce immuno-tolerance in engraftment of allogenic skin.

Category: Early experiments with well-defined objectives/hypotheses

POSTER 21

COMBINATION THERAPY I RECALCITRANT DISCOID LUPUS ERYTHEMATOSUS

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We present 3 patients with refractory cutaneous lupus erythematosus (CLE) with excellent response to combination antimalarials and acitretin. Patient 1, a 50-year old female with systemic lupus erythematosus (SLE) presented in 2001 with widespread discoid lupus erythematosus (DLE). Topical steroids, calcineurin inhibitors, antimalarials, and immunsuppressant's were ineffective. In November 2011 acitretin was added to combination antimalarials with rapid response, sustained at 9-months. Patient 2, a 61-year old male presented in 2003 with DLE affecting the face/scalp. Ineffective therapies included topical steroids, calcineurin inhibitors, antimalarials, dapsone and various immunosuppressant's. Acitretin was added to chloroquine in January 2012 with significant improvement at 11-month follow-up. Patient 3, a 66-year old female with SLE presented in 2004 with a 15-year history of CLE. Combined antimalarials were limited by the development of retinal toxicity and methotrexate was ineffective. Acitretin 10mg daily was commenced in July 2012 with quinacrine with dramatic clearance of all lesions and sustained response at 6-months. In all patients serum lipids and liver function tests remained stable and no significant side effects were reported. Combination antimalarial therapy is beneficial in patients with recalcitrant CLE. In patients in whom anti-malarials fail, additional therapies are required. A recent report demonstrated the successful treatment of recalcitrant DLE with combined antimalarials and acitretin. Our series supports the use of this combination in selected patients with resistant CLE. Furthermore, the beneficial effects of antimalarials on lipid profiles may limit the changes induced by retinoids. We propose a study of the immunomodulatory effects of systemic retinoids in CLE.

Category: Pilot/exploratory experiments (for study design, hypotheses creation, etc)

POSTER 22

POLARIZATION AS A POTENTIAL TOOL FOR SKIN CANCER DETECTION

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We have been investigating the use of laser polarization as a simple and inexpensive means of aiding non-experts in distinguishing lesions, thereby improving care in underserved areas. When polarized lasers interact with skin, polarization reduces. This depolarization can be quantified by the depolarization ratio $D = (I_{horizontal} - I_{vertical})/(I_{horizontal} + I_{vertical})$, where $I_{horizontal}$ and $I_{vertical}$ are the scattered intensities measured through a polarizer oriented in the horizontal and vertical directions with respect to the polarization of the incident illumination. Previous studies demonstrated that depolarization ratios generated from the stationary region of the illuminated field could separate malignant from benign lesions. In this pilot study, we investigated the feasibility of performing skin lesion differentiation by analyzing the distribution of depolarization ratios collected radially from the centre of lesions. We tested our method in-vivo on a set of skin lesions including seborrheic keratosis, nevus, and normal skin. The experimental set-up consists of a laser that emits a narrow beam of horizontally polarized light, which is reflected off skin. The reflection passes through a polar filter, and $I_{horizontal}$ and $I_{vertical}$ are computed as the filter is rotated from horizontal to vertical. The computed depolarization ratio distributions of the tested lesions were quantifiably different. We expect that radial depolarization distributions provide more data for lesion separation then

depolarization ratios alone. This approach may potentially increase the accuracy in diagnosing skin conditions.

Category: Pilot/exploratory experiments (for study design, hypotheses creation, etc)

POSTER 23

CHARACTERIZATION OF THE ROLE OF TUMOR SUPPRESSOR, BIN1, AS A POTENTIAL MEDIATOR OF THE AHI-1 ONCOGENE IN HUMAN CUTANEOUS T-CELL LYMPHOMAS

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Cutaneous T-cell lymphomas (CTCLs) represent a group of lymphoproliferative disorders that are characterized by the homing of malignant T-cells to the surface of skin. Two main types of CTCL are Mycosis Fungoides and its leukemic variant Sezary Syndrome (SS). The precise genetic pathogenesis of these diseases remains largely undetermined. Our group has demonstrated that expression of AHI-1 oncogene is highly elevated in CTCL lines (Hut78 and Hut102), as well as in primary $CD4^+CD7^-$ Sezary cells. Furthermore, stable knockdown of endogenous AHI-1 in Hut78 cells reduces their transforming activity both *in vitro* and *in vivo.* By conducting microarray analysis on AHI-1-suppressed Hut78 cells (AHI-1/sh4), several differentially expressed genes have been identified that might be involved in the same pathways as AHI-1 in SS. One candidate is BIN1 which is upregulated at RNA and protein levels in AHI-1/sh4 cells and is downregulated in primary Sezary cells. BIN1 has more than ten isoforms, some with tumor suppressor activities in solid tumors. However, the role of BIN1 in regulation of normal hematopoiesis and lymphomagenesis remains unknown. The aim of this project is to investigate the role of BIN1 isoforms and their potential molecular connection to AHI-1 in CTCL using both *in vitro* and *in vivo* assays. This project will extend our knowledge of the underlying disease mechanisms of SS and potentially of other types of CTCL. A deeper insight into the roles of specific genes and signaling pathways involved in SS development may eventually identify new molecular targets for more effective treatment options.

Category: Early experiments with well defined objectives/hypotheses

POSTER 24

TOPICAL CPG ADJUVANT ENHANCES IMMUNE RESPONSE TO SUBCUTANEOUS ANTIGEN BY MODULATING THE CUTANEOUS LYMPH NODE ENVIRONMENT

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Current vaccines are mainly administered by intramuscular route but the skin harbors many immune cells and can be used as a site of immunization. Topical CpG oligodeoxynucleotide (ODN), a Toll-like receptor 9 (TLR9) agonist, improves humoral and cell-mediated immune responses to locally injected protein-based vaccines in mice but the mechanisms are still unclear. Topical CpG ODN is internalized by $CD11c^+$ cells that are detected within the skin draining lymph nodes (SLN) at 48 hours post treatment. Ablation of TLR9 in the hematopoietic compartment of bone marrow chimeric mice abrogates antigen-specific $CD8^+$ T cells production. TLR9 ablation in the stromal compartment also decreases topical CpG adjuvant effect. Topical

CpG ODN differentially modulates the environment of SLN compared to subcutaneous administration within 24 hours of treatment (with 4-7 fold increased expression of 4 genes: Ccl4, Cxcl3, Ifng and Il11. A higher proportion of antigen-specific $CD4^+$ T cells in the SLN express tissue-homing molecules (P- and E-selectin ligand) when adjuvant is administered topically compared to subcutaneously. Egress of lymphocytes from SLN is necessary for optimal CpG adjuvant effect using a contact hypersensitivity model. We propose that topical CpG ODN activates stromal cells as well as hematopoietic-derived cells in the skin, to subsequently modulate the environment of SLN. These changes enhance protective humoral and cell-mediated immune responses.

Category: Applied/functional experiments

Poster 25

ANTI-MELANOMA DIFFERENTATION-ASSOCIATED GENE 5 (MDA5) ANTIBODIES AND MEMBRANE ATTACK COMPLEX (MAC) FORMATION IN CLINICALLY AMYOPATHIC DERMATOMYOSITIS (CADM)

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Connective tissue diseases can be challenging to diagnose. One such example is distinguishing between Degos disease, a vasculopathic disorder, and CADM, both of which can have overlapping features. Anti-MDA5 antibodies have been shown to correlate with interstitial lung disease and skin ulceration in dermatomyositis with minimal or no muscle involvement (CADM). MAC has been shown to correlate with dermatomyositis and ischemic skin changes. Anti-MDA5 antibodies from serum and MAC staining from formalin-fixed skin biopsies will be obtained from a series of patients in order to corroborate these findings and for diagnostic clarification.

POSTER 26

EXTRACUTANEOUS MELANOMA: A CANADIAN PERSPECTIVE ON EMERGING EPIDEMIOLOGICAL TRENDS AND COMPARISONS TO CUTANEOUS MELANOMA

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Introduction: Primary extracutaneous melanomas (ECMs) are rare cancers that are often identified at a late stage and have poorly-defined screening strategies. As their epidemiology lacks characterization for the Canadian population, we sought to analyze ECM incidence and survival rates in British Columbia and compare trends to those of cutaneous melanoma (CM). Methods: Data on ECMs and CMs diagnosed between 1992 and 2006 were obtained from the BC Cancer Registry. The ECM cases were divided into anatomical sites based on ICD-9 codes, and incidence rates for each site were age-standardized and separated by sex. CM categories were divided based on body site and histological type. 5-year survival rates were tracked until 2011. Results: A total of 922 ECMs and 17,108 CMs were recorded between 1992 and 2006. Ocular melanomas were the most frequent ECM, with an age-standardized incidence rate (per million) of 10.6 for males and 8.5 for females. ECM patients were generally older at diagnosis and had poorer survival rates compared to CM cases. Survival rates for ECM, however, varied drastically from

23.5% for genital lesions to 87.0% for ocular cases; CM survival rates showed less variation when subcategorized by body site and histological type. Conclusions: Our ECM epidemiology results are largely consistent with previous studies from the U.S. and Europe, and differences in reported values provide opportunities to assess the efficacy of cancer detection, monitoring and treatment strategies in different global regions. Our study lays a foundation for future ECM epidemiological comparisons in both Canada and internationally.

Category: Early experiments with well defined objectives/hypotheses

POSTER 27

ALLERGIC CONTACT DERMATITIS TO LOCAL ANAESTHETICS: REVIEW OF DATA FROM 35 REACTIVE PATIENTS AND CLINICAL IMPLICATIONS.

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Delayed type of hypersensitivity to many commonly utilized local anaesthetics is not that rare amidst patients with clinical manifestations of contact dermatitis presenting for patch testing. This is felt to be largely due to exposure in non-prescription preparations, and is of particular concern in the elderly who are high users, and who at the same time begin to require local anaesthesia on more frequent basis for various medical procedures. Here we present the data from our Contact Dermatitis Clinic from January 2009 to July 2012 on 35 patients who are patch test positive to local anaesthetics. The overall prevalence of allergic contact dermatitis to common local anaesthetics was 2.3% amongst the 1,522 patient in the database. Benzocaine was the most frequent, comprising 45.7% of the positive patch tests. However, lidocaine comprised a greater proportion of the reactions than anticipated, at 28.6%. The proportion of patients who are patch test positive to local anaesthetics and who will truly have peri-operative problems with intra-dermal administration of ubiquitously used amide anaesthetics needs to be established. Likewise, there is no clear data on the clinically significant cross-reactivity between widely available and sensitizing ester topical anaesthetic – benzocaine – and the injectable amide anaesthetic of choice – lidocaine. We intend to examine these queries by challenging the patients who have tested positive to local anaesthetics with intra-dermal injections of lidocaine and assessing for the development of delayed type of hypersensitivity reaction.

Category: Early experiments with well defined objectives/hypotheses

POSTER 28

EFFICACY OF PRP INJECTIONS IN PATIENTS WITH ANDROGENETIC ALOPECIA

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Introduction: Platelet rich plasma is an innovative therapy which gained considerable attention in different medical fields including orthopedics, dentistry and dermatology. In dermatology it has been used mainly in chronic wound treatments because of its tissue healing ability and tested in hair transplantation to improve graft survival. Recently there have been reports supporting its use in hair loss; one published paper supported its efficacy in androgenetic alopecia AGA. PRP works by delivering more than 20 different growth factors and cytokines locally at supraphysiological levels. The study will examine PRP efficacy in AGA which is the most common causes of hair loss overall. AGA treatment varies including minoxidil, 5-

alpha reductase inhibitors and hair transplantation. Each of the above has its own drawbacks. Study Design: 20 AGA patients will be enrolled. Trichoscopy will be used to measure follicle numbers and mean hair shaft diameter in the first visit. In each visit, a fixed blood volume will be drawn to prepare PRP and will be injected intralesionally into a bald patch. Normal saline will be injected into an adjacent similar during the same visit as a control and trichoscopy readings will be taken. Injections will be given every 2 weeks for 10 weeks and patients will be followed for 10 weeks. End point: The change in the mean number of hair per cm2 and the mean shaft diameter compared with control. Inclusion Criteria: Adult males and females, with AGA. Exclusion criteria: Minoxidil and/or 5-alpha reductase inhibitors use within 3 months of study enrollment.

Category: Pilot/exploratory experiments (for study design, hypotheses creation, etc)