Oral Presentations

(8:35am)

TOLL-LIKE RECEPTOR 9 INVOLVEMENT IN SKIN WOUND REPAIR

Alan Hoi Lun Yau, Wing Ki Cheng, Jan P Dutz.
Department of Dermatology and Skin Science, University of British Columbia, Canada.

Background: Toll-like receptors (TLRs) have been implicated in microbial recognition and wound repair. Mice deficient in MyD88 (downstream adapter molecule common to all TLRs except for TLR3) have been shown to exhibit delayed and decreased skin wound repair. TLR-9 expression in keratinocytes has been found to be up-regulated after skin wounding, and it has been suggested that TLR-9 induces angiogenesis to promote skin wound repair. The involvement of TLR-9 in skin wound repair was examined *in vivo*. Methods: Dorsal skin of C57BL/6 control (n = 8), MyD88 deficient (n = 8) and TLR-9 deficient (n = 8) mice was shaved and disinfected with Betadine. Two circular, 4-mm diameter, full-thickness wounds (one on each side of midline) were surgically induced by punch biopsy and covered with a transparent dressing (Tegaderm 3M). Wounds and a calibration scale were photographed using a digital camera for area quantitation (Image Pro 6) every other day over 10 days. Results: No significant difference in the rate of skin wound closure was found among the 3 experimental groups. This could be due to the use of small size wounds (4-mm diameter) which contracted rapidly, masking any actual differences in skin wound closure among the groups. Conclusion: Further experiments that utilize larger size wounds and examine histological events in skin wound repair are needed to confirm the involvement of TLRs. Clinical Significance and KT: Applied and functional experiments that identify receptors involved in tissue injury recognition offer potential therapeutic targets for intervention.

(8:47am)

A PROTECTIVE PROTEIN MAKES PRIMARY SKIN CELLS RESISTANT TO THE APOPTOTIC EFFECTS OF IDO

Darya Habibi[1, 2], Reza B Jalili[1], Farshad Forouzandeh[1], Christopher Ong[2], Aziz Ghahary [1].
[1] Burn and Wound Healing Research Lab, Department of Surgery, University of British Columbia, [2] Prostate Centre, University of British Columbia

Introduction: We have previously used the immunosuppressive effects of Indoleamine 2,3-dioxygenase (IDO), a tryptophan-degrading enzyme, in the development of a non-rejectable skin substitute. We have also shown that IDO expression selectively induces apoptosis in immune cells rather than primary skin cells. However, the mechanism(s) underlying selective resistance of skin cells to IDO exposed environment is not fully elucidated. Here we asked whether the activity of general control nonderepressible-2(GCN2) kinase stress-responsive pathway and its known inhibitor, protein IMPACT

homolog, in immune and skin cells is differentially regulated in response to IDO-induced low tryptophan environment. Methods: IDO-expressing fibroblasts were co-cultured with bystander Jurkat cells, human $CD3^+$ Tcells, fibroblasts or keratinocytes. The levels of phosphorylated GCN2, total GCN2 and IMPACT were evaluated by Western blot analysis. MTT and viability assays were performed for IMPACT siRNA-knocked down fibroblasts co-cultured with IDO-expressing fibroblasts. Results: Activation of GCN2 kinase pathway was significantly higher in immune cells exposed to IDO, relative to that of skin cells. In contrast, IMPACT protein was highly and constitutively expressed in primary skin cells while its expression level was very low in T cells and undetectable in Jurkat cells. A significant IDO-induced suppressive as well as apoptotic effect was demonstrated in IMPACT-knocked down fibroblasts co-cultured with IDO-expressing fibroblasts. Conclusion: High expression of IMPACT in non-immune cells acts as a protective mechanism against IDO-induced GCN2 activation. Clinical Significance and KT: This study revealed that IDO expression can function as a local immunosuppressive factor to protect the allograft skin without compromising skin cell viability.

(8:59am)

CALIBRATING AN INEXPENSIVE SKIN LESION IMAGING SYSTEM

Paul Wighton[1,2,3], Tim K. Lee[1,2,3], David McLean[2,3], Harvey Lui[2,3], M. Stella Atkins[1]
[1]School of Computing Science, Simon Fraser University, Burnaby BC.
[2]BC Cancer Research Centre, Vancouver BC.
[3]Photomedicine Institute, Department of Dermatology and Skin Science, University of British Columbia and Vancouver Coastal Health Research Institute, Vancouver BC

An increase in both the affordability of consumer digital cameras and the popularity of LED based dermoscopes is lowering the barrier to entry of digital dermoscopy for many dermatologists as well as primary health care providers. Such 'low cost' solutions however have several disadvantages. Firstly, the illumination across the field of view is not consistent, resulting in both areas of over- and underexposure within the same image. Secondly, the color of the image acquired is not accurate. Commercial digital cameras are optimized to operate under certain typical lighting conditions (such as sunlight, incandescent light, etc.), and thus have difficulty estimating the properties of unconventional light sources such as those within dermoscopes. Moreover, consumer-grade cameras are more concerned with presenting visually esthetic images, rather than faithful renditions of color per se. Finally, the coupling of the dermoscope and camera lens introduces certain artifacts. The most notable, chromatic aberration, occurs when the refractive indices of the optics are not constant with respect to wavelength. Fortunately these disadvantages can be mitigated via calibration. We present a calibration method that corrects for these three issues (inconsistent illumination, incorrect colour and chromatic aberration). After calibration, illumination which originally varied by 2.5 times is rendered constant, color estimation accuracy becomes

consistent with other published techniques, and distortions due to chromatic aberration are reduced by approximately 74%. Clinical Significant and KT: Applied experiments. The calibration method described can be used to improve the quality of medical skin images acquired using low cost equipment.

(9:11am)

A SYSTEMATIC REVIEW OF ADVERSE CUTANEOUS REACTIONS RESULTING FROM THE USE OF RIBAVIRIN AND INTERFERON

Jonathan Shapero, Nisha Mistry and Richard Crawford
Department of Dermatology and Skin Science, University of British Columbia

Introduction: Interferon and ribavirin have become the gold standard of treatment for chronic hepatitis C. Many of their adverse effects are skin reactions. Methods: A MEDLINE search was conducted for articles from January 2000 to August 2008 under the headings interferon/adverse effects or ribavirin/adverse effects. This search yielded 2599 results. The results were then manually reviewed for those studies detailing dermatologic findings. Results: Local injection site reactions occur in the majority of patients treated with interferon injection. 59% of all non-injection-site cutaneous eruptions secondary to interferon are the "eczematoid" drug reaction. Eczema variants of Meyerson's phenomenon and nummular dermatitis have also been reported. Diffuse hair thinning is estimated to occur in 19% of patients being treated with combination interferon and ribavirin. Less common hair reactions that have been reported are alopecia areata, alopecia universalis, eyebrow and eyelash trichomegaly, generalized hypertrichosis, hair curling, and hair repigmentation. Over 70 cases of sarcoidosis secondary to interferon have been reported, of which at least 30 have primarily involved the skin. Other reported adverse reactions include psoriasis, lupus, fixed drug eruption, hyperpigmentation of the tongue, vitiligo, lichenoid eruptions, apthous ulcers, Grover's disease, delusions of parasitosis, pyoderma gangrenosum, atrophie blanche, dermatomyositis, scleromyxedema, polyarteritis nodosa and rosacea fulminans. Discussion: The range of skin reactions caused by interferon and ribavirin is quite distinct from those of most other medications. This may be due to an unmasking of a predisposition to diseases like psoriasis, eczema, lupus, sarcoidosis and alopecia areata because of pro-inflammatory properties.

(9:23am)

TUMOR SUPPRESSOR ING1B MAINTAINS GENOMIC STABILITY UPON UV-INDUCED REPLICATION STRESS

Ronald PC Wong, Leon H Lin, David W Chen and Gang Li
Department of Dermatology and Skin Science, Jack Bell Research Center, Vancouver Coastal Health Research Institute, University of British Columbia, Vancouver, British Columbia, Canada

Genomic instability plays an important role in cancer development. Human genome is susceptible to genetic alterations such as chromosome rearrangement during replication. Individuals with genetic defects in genes involved in DNA replication and repair pathways are predisposed to cancers. Ultraviolet (UV) irradiation is the major environmental risk factor for skin cancers. UV lesions present on DNA template block progress of replication fork. Prolonged stalling of replication leads to genomic instability and contributes to cancer development. Tumor suppressor Inhibitor of Growth 1b (ING1b) has been shown to be reduced or mislocalized in various cancers. It is shown to be involved in UV response, but the exact role has not been elucidated. In this study, we found that depletion of physiological level of ING1b sensitized cells to UV. ING1b depleted cells exhibited a prolonged S phase arrest and defect in recovery from UV-induced replication blockage. Moreover, ING1b depletion increased H2AX phosphorylation, which is a hallmark for DNA double strand breaks, and formation of aberrant chromosome structures after UV irradiation. Interestingly, ING1b was required for efficient PCNA monoubiquitination, indicating that ING1b may be involved in lesion bypass mechanism. We herein describe a novel tumor suppressive function of ING1b in the maintenance of genomic stability after UV irradiation. Clinical Significance and KT: This study leads to a better understanding of the mechanism involved in preserving genomic stability. It has implications in developing new strategies for skin cancer prevention and detection.

(9:35am)

CXCR3/ LIGANDS PROMOTE BASAL CELL CARCINOMA CELL PROLIFERATION

Blanche K.K. Lo, Mei Yu, David Zloty, Bryce Cowan, Jerry Shapiro, Kevin J. McElwee
Department of Dermatology and Skin Science, University of British Columbia, Vancouver, BC, Canada

Basal cell carcinoma (BCC) is the most common cancer in humans. The small secretory chemokines CXCL9, 10, 11 and their receptor CXCR3, have been found to be associated with several advanced stage cancers. As such, we hypothesized that CXCR3 and its ligands may also be involved in BCC development. In this study, quantitative RT-PCR revealed that *CXCL9, 10, 11* and *CXCR3* were significantly upregulated by 22.6-fold, 9.2-

fold, 26.6-fold, and 4.9-fold respectively in BCC tissue samples as compared to non-lesional skin epithelium. Immunohistochemistry (IHC) revealed that CXCR3, CXCL10 and CXCL11 colocalized with the BCC marker, cytokeratin 17 (K17), in tumor masses. Cells were isolated from human nodular BCC tissues and were treated with CXCL11 peptide under various concentrations and time-points. Treatment with 10nM CXCL11 was identified as the optimal concentration with a statistically significant increase in cell numbers by day 21 of culture. Dual-label IHC of the treated BCC cells revealed that on day 21, both $K17^+/CXCR3^+$ and $K17^+/CXCR3^-$ cell groups (65.47% and 33.09% respectively) significantly dominated the cell population. The expression of CXCR3 and its ligands in human BCC keratinocytes and the CXCL11 supplementation leading to $K17^+$ BCC cell proliferation *in vitro*, suggest that CXCR3 and its ligands may be important autocrine and/or paracrine mediators for BCC growth. Clinical significance and KT: our findings suggest suppression of CXCR3 chemokine signaling may be a novel strategy for BCC treatment.

(9:47am)

BACK ATLAS FOR TRACKING PIGMENTED SKIN LESIONS

Hengameh Mirzaalian[1,2], Ghassan Hamarneh[1], Tim K. Lee[1,2,3]
[1]Medical Image Analysis Lab, Simon Fraser University, BC Canada; [2]Photomedicine Institute, Department of Dermatology and Skin Science, University of British Columbia and Vancouver Coastal Health Research Institute, BC Canada; [3]Cancer Control Research, BC Cancer Agency, BC Canada.

Tracking pigmented skin lesions (PSL) is commonly performed for high risk melanoma patients. Patients are photographed to allow physicians to periodically compare the images taken at different times in order to detect changes in the number of PSL and their appearance. Recently, we initiated a study to automate the tracking of PSL from images of human backs. In computer vision, the state-of-the-art approach for comparing images is to perform spatial normalization by warping images into a common coordinate frame of reference, an atlas. As an example, a brain atlas allows us to compare data from different subjects or from the same subject taken at different times. Inspired by such works, we propose the first atlas of the human back, which is a unit-square patch with a set of longitudes and latitudes anchored by anatomically meaningful landmarks. Back images taken at different times are also divided into grids of longitudes and latitudes. Then, the grids along with PSL are warped to the proposed atlas, where matching is performed. We evaluated our back atlas by registering 56 pairs of back images using several PSL matching algorithms with and without an atlas. Our experiment showed that the anatomy-based atlas improved the matching accuracy by 2% to 50%. Clinical Significance and KT: Automating the tracking process can help detect changes in PSL, which is critical for early detection of potential malignancies. In this applied experiment, we proposed the first human back atlas and showed that it could substantially improve the matching accuracy of PSL.

(11:05am)

NQO1 INDUCES THE PROLIFERATION OF MELANOMA CELLS

Marco Garate, Aijaz Wani and Gang Li.
Department of Dermatology and Skin Science, Jack Bell Research Centre, Vancouver Coastal Health Research Institute, University of British Columbia, Vancouver, British Columbia, Canada.

The NAD(P)H:quinone oxidoreductase (NQO1) plays a prominent role in maintaining the cellular homeostasis. This ubiquitous oxidoreductase catalyzes the metabolism of quinones and is induced along a battery of defensive genes to provide resistance for cells against oxidative stress and therefore is considered a cell protecting agent against this stress. On the other hand, NQO1 is overexpressed in many tumors and it has been suggested that in melanoma cells NQO1 can activate the nuclear factor kappa B (NF-kB) and in turn induce cell proliferation. Working with a number of cell lines we have previously shown that NQO1 is overexpressed in most melanoma cell lines when compared to melanocytes and the expression of NQO1 is associated with faster proliferation and cell cycle progression. In the current work, we hypothesized that NF-kB mediates the effect of NQO1 in melanoma cell proliferation. Using NQO1 deficient and overexpressing cells, we found that NQO1 stabilizes the B-cell lymphoma 3-encoded protein (BCL3) a co-activator that induces transcription through association with NF-kB p50 homodimers. Through BCL3 and NF-kB p50 knockdown experiments, we found that BCL3 mediates the effect of NQO1 on NF-kB by inducing the expression of p50 and its precursor p105. In turn, the upregulation of NF-kB p50 induces melanoma cell proliferation. These results suggest that NQO1 plays a key role in melanoma pathogenesis. Clinical Significance: This study unveils the molecular mechanism of how NQO1 induces melanoma progression and therefore may lead to the design of more effective therapies for melanoma patients.

(11:17am)

FLUORESCENCE EXCITATION-EMISSION MATRIX (EEM) SPECTROSCOPY FOR SKIN AUTOFLUORESCENCE CHARACTERIZATION

Jianhua Zhao, Florina Feng, Martin Tsai, Haishan Zeng, David I. McLean and Harvey Lui
Laboratory for Advanced Medical Photonics, Photomedicine Institute, Department of Dermatology and Skin Science, University of British Columbia & Vancouver Coastal Health Research Institute, and Integrative Oncology Department, BC Cancer Research Center

Background. A number of endogenous skin fluorophores have been identified, including tryptophan, keratin, collagen, elastin, melanin, NAD(P)H and FAD. Some physiological and pathological processes such as aging, photoaging, psoriasis and skin

cancers have shown characteristic changes in skin fluorescence. However, previous studies were either limited in terms of the excitation or emission wavelengths used or the specific body sites assessed. Variations in skin fluorescence due to anatomic location have never been systematically examined by EEM spectroscopy, which scans the excitation and emission wavelength and can provide the full fluorescent characteristics of the skin. Methods and Results. We studied the EEM skin properties for 10 anatomic skin sites using a double-monochromometer based spectrofluorometer. The measurement sites include forehead, cheek, nose, neck, palm, thumbnail, dorsal surface of hand, dorsal surface of forearm, medial surface of arm and mid-back. The excitation and emission wavebands were 260 - 450 nm and 300 - 700 nm with 5 nm steps, respectively. Twenty patients were recruited for this study. The optimal excitation/emission wavelengths of major skin fluorophores were identified. We found that facial skin has strong tryptophan and porphyrin fluorescence; palm and nail have strong tryptophan and collagen/elastin fluorescence; and other body sites have strong collagen/elastin fluorescence. Conclusions. Apparent differences can be easily determined from EEM spectra of these body sites. Clinical Significance and KT. This study belongs to the category of applied/functional experiments. The results help the understanding of skin morphology and chemical compositions and may eventually help improve skin disease diagnosis.

(11:29am)

KERAKINOCYTE-RELEASABLE FACTOR INCREASES EXPRESSION OF MMP-2, MMP-3 AND INDUCES EXPRESSION OF COLONY STIMULATING FACTOR- 3 IN FIBROBLAST.

Moeen Rezakhanlou A, Li M, Nabai L and Ghahary A.
BC Professional Fire Fighters' Burn and Wound Healing Laboratory, Department of Surgery, Jack Bell Research Centre, University of British Columbia, Vancouver, BC, Canada.

Introduction: Interaction of two major cells in skin, Keratinocyte and Fibroblast, plays significant role in skin integrity in health and pathologic circumstances. Cross talking of Keratinocyte and Fibroblast takes place by modulation of production of multiple proteins. Based on our previous publications and other data, we hypothesize that the fibroblast and keratinocyte cross-talking with each other by secreted proteins such as cytokines and MMPs. Method: We co-cultured the two different cells types for 48 hrs and isolated RNAs and examined expression of 110 different cytokines at transcription level by microarray. We also used RT-PCR and western blotting techniques to confirm the microarray results. In addition, we examined the expression level of MMPs and cytokines in condition media. Results: The cytokine array, RT-PCR and western blotting results showed that keratinocyte induces de novo synthesis of Colony Stimulating Factor 3 (CSF-3) in fibroblast in co-culture condition. The high level of CSF-3 expression has been detected in transcription level and in condition medium. We also showed that

MMP-2, MMP-3, Fibronectin are produced by fibroblast in co-culture with Keratinocyte. Conclusion: The results indicate that human keratinocyte and fibroblast release some proteins which modulate extracelluar matrix proteins such as MMPs and fibronectin. These proteins play significant role in wound healing and scar remodeling. In addition, colony stimulating factor-3 is secreted from fibroblast in response to keratinocytes stimulation, has major effect on proliferation, maturation and activation of neutrophils.

(11:41am)

THE MOON CHILDREN OF KUNA YALA: ALBINISM IN SAN BLAS ISLANDS OF PANAMA. REVIEW, DIRECTIONS IN RESEARCH AND AID.

Irèn Kossintseva, Jan P Dutz.
Department of Dermatology and Skin Research, University of British Columbia, Vancouver, BC, Canada

The Kuna Indians are a small indigenous population in Central America, which own the province of Kuna Yala that spans 400 miniature San Blas Islands off the east Panama coast. Their island population of about 30,000 resides in compact quarters, inhabiting only a few of those islands because they value tightly-knit community over independent personal spread. The Kuna Indians have the world's highest rate of oculocutaneous albinism (specifically OCA2), with current estimated frequency of 1:160. The albinos, called the Moon Children, play a central role in Kuna mythology, are revered to have special powers and subsequently are often rendered prime positions in the community. While an appreciable anthropological data has been gathered about the Kuna albinism, there has been surprisingly minimal medical research. This is likely in part due to relative impermeablility to Westernization by this proud and hard-working community. The location of San Blas Islands archipelago in the equatorial belt predisposes the Kuna albinos to the morbidity and mortality associated with solar insult. Here we present a review of physical anthropology and medical data on the Kuna albinism to date, along with personal experience and photographical record of these Moon Children afflicted from a very young age with actinic keratoses and morphology consistent with squamous cell carcinoma. We propose a project for questionnaire-based research on the understanding of and attitudes towards albinism, sun avoidance and skin cancer amongst the Kuna albino population. Further, we hope to establish on the basis of our findings, a sun-safe albino outreach skin-care program.

(11:53am)

BRG1 EXPRESSION IS INCREASED IN HUMAN CUTANEOUS MELANOMA

Leon Hanyang Lin,[1] Ronald Pak Cheung Wong,[1] Michal Martinka,[1] Magdalena Martinka,[2] and Gang Li[1]
[1]Department of Dermatology and Skin Science, [2]Department of Pathology, Jack Bell Research Centre, Vancouver Coastal Health Research Institute, University of British Columbia, Vancouver, British Columbia, Canada

The SWI/SNF chromatin remodeling complex plays essential roles in a variety of cellular processes including differentiation, proliferation, and DNA repair. Aberrant expression of SWI/SNF chromatin remodeling complex is involved in cancer development. The core subunit of SWI/SNF complex, SNF5, is found deleted in malignant rhabdoid tumors. Our recent published data also showed that SNF5 is down-regulated in melanoma. Although the catalytic subunit of SWI/SNF complex, BRG1, is also found inactivated in many lung, breast, and prostate cancer cell lines, experimental observations suggest that SNF5 and BRG1 do not have the same functions. To investigate the putative role of BRG1 in the development of melanoma, we examined the expression of BRG1 in melanocytic lesions at different stages and analyzed the correlation between BRG1 expression and clinicopathologic variables and patient survival. Using tissue microarray and immunohistochemistry, we evaluated BRG1 staining in 48 dysplastic nevi, 90 primary melanomas, and 47 metastatic melanomas. Surprisingly, BRG1 expression was increased in primary melanoma compared with dysplastic nevi ($P < 0.0001$), and in metastatic melanoma compared with dysplastic nevi ($P < 0.0001$), but not in primary melanoma compared with metastatic melanoma. Furthermore, we showed that knockdown of BRG1 in human melanoma cell lines reduced cell proliferation. Our data indicate that BRG1 may play a role in early stage of melanomagenesis. KT category: (2) Early experiments with well defined objectives/hypotheses.

(12:05pm)

CHARACTERIZATION OF PLP1 EXPRESSING CELLS IN HUMAN SKIN

Richard Yu, Youwen Zhou
University of British Columbia, Department of Dermatology and Skin Science, Vancouver, BC, Canada

Background: PLP1 is a transmembrane proteolipid protein that forms the myelin sheath in the mammalian nervous system. Recently, it has been found that this gene is dramatically decreased in the lesional skin of vitiligo, a skin condition characterized by the death of melanocytes, raising the possibility that their death is the cause of PLP1 decrease. Objectives: The purpose of this study is to evaluate expression of PLP1 in cultured normal human epidermal melanocytes and in the skin biopsies of human normal skin. Methods: Normal human epidermal melanocytes were cultured and

harvested for determination of PLP1 messenger RNA expression using other cell types as the control. In addition, immunofluorescence studies against PLP1 were performed to detect cells and structures in the human skin biopsies, using other markers such as CD56/NCAM, neurofilament and Melan-A as the controls. Results: Cultured human melanocytes express PLP1 mRNA, but less compared with peripheral skin nervous tissues. Further, in human skin biopsies, epidermal melanocytes do not express detectable levels of PLP1 protein. The main locations of the cells that express PLP1 protein in human skin are located in the superficial dermis, some in close proximity to the epidermal melanocytes. Conclusion: The main sources of PLP1 expression in human skin is not likely the melanocytes. Rather, they are the superficial dermal nervous cells, most likely the Schwann cells. Clinical Significance and KT: The result of this study leads to the speculation that vitiligo not only involves the death of melanocytes, but also defects in the dermal nervous system.

(12:17pm)

WITH A CLINICAL SUSPICION OF MELANOMA, DOES THE TYPE OF BIOPSY IMPACT THE EFFICIENT USE OF RESOURCES AND ABILITY OF THE PATHOLOGIST TO PROVIDE THE PERTINENT INFORMATION FOR APPROPRIATE MANAGEMENT OF THE PATIENT?

Chris Sladden, Richard I. Crawford
Department of Dermatology and Skin Science, University of British Columbia, Vancouver, BC.

The gold standard of melanoma diagnosis is histological examination. The pathologist's report confirms or refutes the diagnosis of melanoma, and provides information which can impact treatment, staging and prognosis. Various biopsy types are submitted for histological examination. Using criteria from the pathologist's report and the need for a second Dermatopathology opinion as a measure of worth of the biopsy, samples with suspicion of melanoma/atypical nevus submitted as formalin-fixed tissue or slide consultations over 18 consecutive months to the St. Paul's Hospital pathology department were reviewed. 937 samples encompassing the range of biopsy types and specialty of the submitting physicians were reviewed. 536 excision-biopsies included 198 melanomas and 338 other diagnoses. One sample could not provide a definitive diagnosis. One melanoma specimen could not provide definitive treatment and prognosis information. 369/534 samples required a second opinion. 173 punch-biopsies diagnosed 49 melanomas and 35 other lesions. 87 specimens allowed no definite diagnosis. Under half of the melanoma samples enabled the pathologist to provide the appropriate information. 159/173 samples required second opinion 78 shave-biopsies included 30 melanomas, 20/30 providing full information. 43 other lesions (5 non-diagnostic) were included. 46/78 samples needed a second opinion. From our study the type of biopsy submitted for pathology impacts the quality of the resulting information and thus the management of patients with pigmented lesions.

(1:20pm)

ROLE OF TUMOR SUPPRESSOR ING4 IN HUMAN MELANOMA

Jun Li and Gang Li
Department of Dermatology and Skin Science, University of British Columbia, Vancouver, British Columbia, Canada

Melanoma is the most lethal form of skin cancer with a high mortality rate due to rapid metastasis. We have previously reported that the expression of ING4, a member of Inhibitor of Growth tumor suppressor family, is significantly reduced in malignant melanoma biopsies compared with dysplastic nevi. Reduced ING4 expression is closely correlated with tumor thickness, ulceration and poor 5-year survival of primary melanoma patients. In this study, we demonstrated that overexpression of ING4 suppressed melanoma cell migration by 63% when compared with the empty vector control, and inhibited RhoA activity and Rock-mediated formation of stress fiber in melanoma cells. Moreover, we showed that overexpression of ING4 inhibited melanoma cell invasion by 46% compared with vector control and significantly decreased matrix metalloproteinase-2 (MMP-2) and MMP-9 activity by 25% and 61%, respectively. Furthermore, we found that ING4 overexpression decreased the expression of interleukin-6 (IL-6) in melanoma cells at both transcriptional and translational level thus inhibited the proliferation and blood tube formation ability of human umbilical vein endothelial cells (HUVEC). Our experiments also showed that ING4 overexpression suppressed the binding activity of NF-kB and NF-kB knockdown abrogated the negative effect of ING4 on HUVEC growth. Finally, in vivo matrigel plug assay demonstrated that ING4 overexpression inhibited the formation of blood vessel in a mouse model. Clinical significance and KT: This study indicated that ING4 plays critical roles in melanoma progression through inhibiting cell migration, invasion and angiogenesis of melanoma, thus it can be potentially used as a new therapeutic target.

(1:32pm)

TOPICAL APPLICATION OF STRATIFIN-EMULGEL REDUCES HYPERTROPHIC SCARRING IN RABBIT EAR FIBROTIC MODEL

Elham Rahmani-N, Reza Jalili, Aziz Ghahary
Burn & Wound Healing Research Lab. Division of Plastic Surgery, University of British Columbia, Vancouver, BC, Canada

Introduction: Hypertrophic scarring (HSc) is a dermal hyperproliferative disorder with excessive accumulation of extracellular matrix (ECM) components that may appear in areas of burn and trauma. It was shown by our group that Stratifin stimulates the expression of matrix metalloproteinases (MMPs) in cultured dermal fiboblasts. Our preliminary experiments showed that a carboxymethyl cellulose (CMC)-hydrogel formulation of Stratifin reduces HSc when topically applied in rabbit ear fibrotic model

in twice-daily regimen. The goal of this study was to modify the CMC-hydrogel formulation in order to reduce the frequency of application, which will enhance patient compliance. Methods: The CMC-hydrogel was modified to an emulgel formulation using a thermoreversible emulsifier and appropriate oil phase. The Stratifin-emulgel (0.01) was applied once-daily on 8-mm circular full thickness wounds created on ventral side of New Zealand white rabbit ears. Thereafter, the quality of wound healing and HSc formation was evaluated. Results: The emulgel formulation created a protective film on the surface of wound due to its thermoreversible characteristic. Wound assessments showed a significantly enhanced MMP-1 expression and reduced scar hypertrophy, epidermal thickness, tissue cellularity and collagen deposition in stratifin-treated wounds compared to the controls. Discussion: In this clinically relevant fibrotic model, wounds treated once daily with Stratifin-emulgel demonstrated a significant reduction in scar hypertrophy. This observation could be the result of increased breakdown of ECM components and reduced tissue cellularity. Clinical Significance: The findings of this study will ultimately result in development of a novel strategy for prevention of HSc following dermal injuries.

(1:44pm)

QUANTITATIVE DISCRMINATION OF PIGMENTED SKIN LESIONS USING NEAR-INFRARED (NIR) FLUORESCENCE IMAGING

Serena Siow, Shuang Wang, Jianhua Zhao, Haishan Zeng, David McLean, Harvey Lui
Photomedicine Institute, Department of Dermatology and Skin Science, University of British Columbia and Vancouver Coastal Health Research Institute; and Integrative Oncology Department, BC Cancer Research Center, Vancouver, BC, Canada.

Background: Cutaneous melanin exhibits strong fluorescence under near-infrared (NIR) illumination. Pigmented skin lesions can be imaged using a prototype *in vivo* NIR fluorescence device. Our objectives are (i) to quantitatively evaluate NIR fluorescence intensities of pigmented skin lesions; and (ii) to define the relationship between NIR fluorescence intensity and melanin content. Methods: A prospective cohort of patients with pigmented skin lesions was recruited. NIR fluorescence images were captured using our prototype NIR fluorescence imaging device. Diffuse reflectance spectra were collected to quantify visual luminosity (CIELAB L*) and melanin content. Results: Over a two-month period, images of 78 pigmented skin lesions were captured. When controlled for normal surrounding skin, mean relative fluorescence ratios were significantly different amongst certain pigmented lesion groups ($p < 0.001$). Mean fluorescence was higher in atypical nevi (mean [95% CI] = 1.37 [1.13 – 1.60]), acquired melanocytic nevi (1.32 [1.24 – 1.40]), and seborrheic keratoses (1.53 [1.35 – 1.71]), and lower in vascular lesions and vitiligo (0.90 [0.73 – 1.08] and 0.82 [0.70 – 0.94] respectively). Regression analysis identified a linear relationship between NIR fluorescence and melanin content both *in vivo* ($r = 0.441$, $p = 0.013$) and *in vitro* ($r=0.937$, $p=0.006$), whereas no specific relationship was observed with luminosity. Conclusion:

We confirm that NIR fluorescence intensities vary among lesion types. NIR fluorescence is directly related to melanin content instead of luminosity. Results from our exploratory experiment provide insight for an objective and direct *in vivo* method of imaging abnormal melanin-containing lesions in the skin.

(1:56pm)

HYPERLIPIDEMIA ACCELERATES SKIN AGING AND DISEASE IN APOLIPOPROTEIN E KNOCKOUT MICE

Paul R. Hiebert[1,2], Thomas Abraham[1], Sara Pazooki[1], Wendy A. Boivin[1,2], Hongyan Zhao[1], David J. Granville[1,2]

[1] Providence Heart + Lung Institute at St. Paul's Hospital, [2] Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

Apolipoprotein E knockout (apoE-KO) mice are widely used as a model of hyperlipidemia-induced atherosclerosis. Concurrent to vascular complications, apoE-KO mice display an aged and diseased skin phenotype. The purpose of this study is to further characterize the role of hyperlipidemia in skin aging and disease. Wild type C57BL/6 and apoE-KO mice were grown to 0, 5, 15 or 30 weeks on either a regular chow diet or a high fat diet. H&E, picrosirius red and luna stains were used to assess morphology and extracellular matrix (ECM) changes in formalin fixed skin sections. Multi-photon microscopy was also used to examine collagen organization in *ex vivo* mouse skin samples. Staining for CD3+ cells, mast cells and the serine protease granzyme B was also performed. ApoE-KO mice demonstrate frailty, hair loss and increased morbidity compared to wild type controls. A reduction in skin thickness, along with marked differences in collagen and elastin were observed in apoE-KO mice compared to wild type controls. Elevated lymphocyte infiltration as well as granzyme B expression was also observed in the skin of apoE-KO mice. These observations were augmented with age and when mice were fed a high fat diet. In conclusion, hyperlipidemia accelerates aging and disease of the skin in apoE-KO mice through increased ECM degradation, possibly due to increased inflammation and proteolytic activity. Clinical Significance and KT: These applied/functional experiments help to understand the effects of hyperlipidemia on the skin thereby promoting better lifestyle choices for those suffering from premature aging or diseased skin.

(2:08pm)

CRYPTOCOCCUS GATTII OUTBREAK IN BC: IN VITRO SUSCEPTIBILITY TESTING TO EIGHT ANTIFUNGAL DRUGS

Nisha Mistry[1], Kennard Tan[2], Sultana Mithani[3], Jeff Fuller[4], Mohammad Shokravi[1], Linda Hoang[3]

[1]Department of Dermatology and Skin Science, University of British Columbia; [2]Department of Laboratory Medicine, University of British Columbia; [3]Bacteriology & Mycology Program, BC Centre for Disease Control; [4]Department of Laboratory Medicine &Pathology, University of Alberta.

BACKGROUND: An ongoing outbreak of *Cryptococcus gattii* in British Columbia (BC) in the immunocompetent population has led to continued focus on this emerging pathogen. Clinical presentation includes pulmonary, neurological and skin findings. Biopsy of cutaneous lesions in cases occurring in 2000 and 2004 allowed for tissue diagnosis of *C. gattii*. Preliminary work on its antifungal susceptibility profiles has been done to date. OBJECTIVE: To assess a commercial antifungal susceptibility testing kit for clinical isolates of *C. gattii* from BC. METHODOLOGY: Minimum inhibition concentrations (MICs) of eight antifungal drugs were determined using the Sensititre YeastOne antifungal panel. Broth microdilution (BMD) (CLSI M38) was used as the reference method. RESULTS: Using interpretive CLSI guidelines for *Candida spp*, all 15 isolates were susceptible to amphotericin B, flucytosine, fluconazole, itraconazole and voriconazole, but resistant to caspofungin. Interpretation guidelines were not available for ketoconazole and posaconazole, although MICs were low. Comparison with BMD found that MIC ranges were consistent for amphotericin-B, fluconazole and caspofungin. However, the MICs for flucytosine and the other azoles measured by YeastOne were low with up to a five-fold $\log_2$ dilution difference. CONCLUSION: For *C. gattii*, the Sensititre YeastOne may be a suitable kit for testing amphotericin B, fluconazole and caspofungin, but not other antifungals. The clinical isolates of *C. gattii* show high susceptibility to itraconazole, ketoconazole and voriconazole, and resistance to caspofungin. CLINICAL SIGNIFICANCE AND KT: Furthering knowledge on the antifungal treatment of choice in patients infected with *C. gattii*.

(2:20pm)

LOOKING INTO THE SKIN WITH *IN VIVO* MICRO-RAMAN SPECTROSCOPY AND REFLECTANCE CONFOCAL IMAGING

Hequn Wang[1,2], Naiyan Huang[1,2], Jianhua Zhao[1,2], Harvey Lui[1,2], Mladen Korbelik[1], Haishan Zeng[1,2]

1) Integrative Oncology Department, British Columbia Cancer Research Centre, Vancouver, B.C., Canada and 2) Laboratory for Advanced Medical Photonics, Photomedicine Institute, Department of Dermatology and Skin Science, University of

British Columbia and Vancouver Coastal Health Research Institute, Vancouver, B.C., Canada.

Skin cancer is the most common type of cancer in North America. Currently biopsy remains the gold standard for diagnosis. Optical techniques could potentially obviate many of the problems that traditional biopsy methods suffer from and offer physicians a non-invasive, high-resolution morphological and biochemical analysis of lesions in real time. The confocal principle performs non-invasive depth-resolved tissue evaluation based on its optical sectioning capability. Raman spectroscopy measures molecular vibrations and offers fingerprint-type specific information for molecular identification. Our goal is to combine these two techniques to achieve non-invasive depth-resolved biochemical analysis of the skin *in vivo* for improving skin cancer detection and evaluation. We built a confocal Raman system consisting of a micro-Raman spectrometer module for measuring Raman spectra of the skin *in vivo* and a reflectance confocal imaging module for guiding the spectral measurements. We conducted a pilot study using a murine squamous cell carcinoma model. Obvious changes in Raman spectra for both the epidermal and dermal layers between normal and tumor-bearing skin were found and these differences could be used to differentiate cancer from normal skin with very high diagnostic sensitivity and specificity. Preliminary confocal images and Raman spectra obtained from both skin samples and healthy volunteers will be presented at the conference. Clinical Significance and KT: We believe that this system will not only improve the non-invasive clinical diagnosis of skin cancer, but also help delineate tumor margins as an aid for treatment planning. It will also enable monitoring of other skin diseases and study of transdermal drug delivery.

(2:32pm)

TOLL-LIKE RECEPTOR 9 EXPRESSION ON HEMATOPOIETIC-DERIVED CELLS IS REQUIRED FOR THE ADJUVANT EFFECT OF TOPICAL CPG ADJUVANT UPON SUBCUTANEOUS OR INTRAMUSCULAR PROTEIN VACCINE ADMINISTRATION

Wing ki Cheng and Jan P Dutz
Department of Experimental Medicine, Department of Dermatology and Skin Science, Child & Family Research Institute, University of British Columbia, Vancouver, BC, Canada

Current vaccine technologies are characterized by a poor ability to induce effective antiviral immunity mediated by cytotoxic T lymphocytes (CTLs). Topical administration of immunostimulatory CpG oligodeoxynucleotide (ODN) 1826, a Toll-like receptor 9 (TLR9) agonist, induced higher level of CTL responses to subcutaneous or intramuscular injected protein vaccines with lower level of toxicity, when compared to subcutaneous or intramuscular injection in a mouse model. TLR9 is expressed in the skin by bone marrow-derived dendritic cells, and by stromal keratinocytes. Since the subset of cells

critical for the adjuvant response remain unknown, bone marrow chimeric C57Bl/6 mice with either the hematopoietic-derived cells or the stromal cells deficient in TLR9 expression were generated. TLR9 expression on hematopoietic-derived cells is required for the topical adjuvant effect of CpG ODN as revealed by abrogated priming of CD8+ antigen-specific T cells in chimeric mice that lack TLR9 expression on hematopoietic-derived cells compared to chimeric mice that were TLR9 sufficient in both types of cells ($0.3 \pm 0.0\%$ vs. $9.8 \pm 0.8\%$, $P<0.0001$). Chimeric mice with stromal cells deficient in TLR9 expression generated fewer antigen-specific CTLs ($1.9 \pm 0.4\%$ vs. $9.8 \pm 0.8\%$, $P<0.0001$) indicating they also contribute to the adjuvant effect of topically applied CpG ODN. Strategies targeting topical CpG adjuvant to both bone marrow-derived and stromal cutaneous cells may optimize the adjuvant effect of CpG ODN. Clinical Significance and KT: Topical CpG ODN administration may increase the efficacy of protein antigen vaccines without a need for re-formulating or re-licensing of current vaccines.

(2:44pm)

MELANOMA INFLAMMATORY RESPONSE GENOTYPING (MIRG)

Irèn Kossintseva[1], Michael Smylie[2], Phil Halloran[3], Michael Mengel[3], Kelly Dabbs[4].
[1]Department of Dermatology and Skin Research, Vancouver, BC; [2]Cross Cancer Institute, Edmonton, AB, [3]Heritage Medical Research Centre, University of Alberta, Edmonton, AB, [4]Misrechordia Hospital, Edmonton, AB.

Malignant melanoma is an aggressive cancer with very little response to the few available treatment options. In the recent years, CTLA-4 antibody therapy has shown encouraging results with some patients achieving long term remission when used as a treatment in Stage III and IV malignant melanoma patients. Because it is an immune-based therapy, it is not yet clear what aspect of melanoma immunology determines the response to this treatment. If this response could be quantified, it would help in determining at an earlier stage which patients would be responsive to this treatment modality. We hypothesize that malignant melanoma expresses a “stereotyped inflammatory response signature” which can be decoded by quantitative genomic analysis using microarray technology. This difference in inflammation, or the inflammatory fingerprint of malignant melanoma subtypes, can be graded molecularly and possibly will aid in predicting clinical response to immune-based therapies. In particular, by doing a class comparison study, it will hopefully help to explain on a molecular level the difference between malignant melanoma patients who are responders and who are non-responders to CTLA-4 antibody (Ab) treatment.