Oral Presentations

(8:30am)

DEVELOPMENT AND APPLICATION OF NON-REJECTABLE SKIN SUBSTITUTE AS WOUND COVERAGE

Farshad Forouzandeh [1], Reza B. Jalili [1] , Steven Boyce [2] , Dorothy Supp [2] , and Aziz Ghahary [1]. [1]Department of Surgery, University of British Columbia. [2]Department of Surgery, University of Cincinnati

Introduction: Any non-autologus grafts will be rejected by the host immune system unless an immunosuppressive agent is used to prevent this rejection. Thus, the goal of this study was to explore a new approach to develop a non-rejectable skin substitute equipped with local immunosuppressive factors such as indoleamine 2, 3- dioxygenase (IDO). Methods: Neonatal foreskin pieces were used as source of fibroblasts and keratinocytes. Constructing IDO-recombinant adenoviruses, we infected fibroblasts. Inoculating the IDO-treated and non IDO-treated fibroblasts into the collagen matrix, we developed the dermal layer. By seeding the keratinocytes on top of this construct, we developed a bi-layer reconstructed human skin. Then, we applied these different skin substitutes on the same size full-thickness skin wounds created on back of Sprague Dawley rats. Thereafter, the wound closure and healing process were evaluated. Results: The finding of this study showed a significant improvement in wound closure time in wounds receiving IDO-expressing skin substitute compared to those of the controls ($P<0.05$). In addition, there was less infiltration of T cells in the IDO group compared to the other groups. Interestingly, more clusters of red blood cells and endothelial cells were present within the grafted areas in the IDO group versus the others. Discussion: Promoting the wound closure, suppressing the immuno-reaction to a non-autologus skin substitute, and inducing angiogenesis are valuable results of using IDO expression in this novel approach to improve wound healing process.

(8:42am)

EXTRAMAMMARY PAGET'S DISEASE: A REVIEW OF THE PATHOGENESIS, DIAGNOSIS AND TREATMENT

Katie Beleznay[1], BSc; Michael Levesque[2], MD; Sharlene Gill[2], MD; John Amiss[3], MD; Anthony Lane[4], MD. 1 – Faculty of Medicine, University of British Columbia. 2 – Faculty of Medicine, Division of Medical Oncology, University of British Columbia. 3 – General and Cosmetic Dermatology, Victoria, BC. 4 – Medical Arts Centre and Nanaimo Regional General Hospital, Nanaimo, BC.

There is limited information in the literature regarding metastatic extramammary Paget's disease (EMPD). This project serves to review the literature on pathogenesis, diagnosis, treatment options and outcomes. EMPD is thought to result from two different pathologic processes. The most common process is EMPD begins as a primary adenocarcinoma in situ that involves the epidermis and adnexa. Less commonly, an underlying adenocarcinoma can extend into the epidermis of the skin leading to secondary EMPD. The common immunohistochemical markers used to diagnose EMPD are simple epithelial type keratins (CK7), sweat gland antigens (CEA, GCDFP-15) and S-100 which is a marker for melanoma. Primary EMPD expresses sweat gland markers (CK7+/CK20-

/GCDFP15+) which is different from secondary which has an endodermal phenotype (CK7+/CK20+/GCDFP15-) and is associated with distant carcinomas. Various therapeutic options have been used for local EMPD. The best results and lowest rates of recurrences can be found with Mohs micrographic surgery. Topical imiquimod has been successfully used in a small number of cases. The occurrence of invasive EMPD has been disputed for a long time. Recently it has been accepted that EMPD can, with time, metastasize. Metastatic EMPD is frequently treatment resistant and has a very poor prognosis. The information on effective chemotherapy and radiotherapy is limited when treating more distant sites such as liver, lung and bone. This project examines these questions and analyzes results to help recognize and manage this potentially deadly disease.

(8:54am)

TOPICAL RESIQUIMOD IS AN EFFECTIVE INDUCER OF CTL TO PARENTERAL ANTIGENS IN MICE

Brent A Chang, Hossain M Najar, Jan P Dutz. Child and Family Research Institute, Department of Dermatology & Skin Science, University of British Columbia.

Generation of effective Cytotoxic T Lymphocyte (CTL) mediated immune responses remains a hurdle for current vaccine methodology. The skin represents a target with strong potential for vaccine strategies which aim to promote CTL priming. We explored the topical use of the Toll-like Receptor (TLR) 7/8 agonist, resiquimod (R848), to enhance cross-priming to subcutaneously administered antigen in a murine model. We show that topical resiquimod can be used to generate robust antigen specific CTL as detected by tetramers, IFN-γ ELISPOT and standard cytotoxicity assays. We also show that topical administration does not require disruption of the skin through tape stripping in order to generate CTL. These CTL are capable of mediating antigen specific killing *in vivo* as measured by *in vivo* cytotoxicity assays and an ability to protect against antigen-bearing tumor challenge. We also observe that multiple applications of topical resiquimod could improve acute CTL priming compared to single applications. The percentage of antigen specific CD8+ T cells in the spleen was higher with multiple applications as measured by tetramer staining and IFN-γ ELISPOT when compared to single application. However, the effects of multiple resiquimod treatment also include other systemic effects, particularly splenomegaly and an apparent shut-down of systemic pro-inflammatory cytokine production.

(9:06am)

TUMOR SUPPRESSOR ING1B REGULATES PCNA MONO-UBIQUITINATION AND S PHASE RECOVERY AFTER UV IRRADIATION THROUGH CHROMATIN REMODELING

Ronald Pak Cheung Wong, Leon Hangyang Lin, 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.

Unrepaired DNA damage would stall replicative DNA polymerases which have stringent requirement for DNA template during replication. The blocked replication fork is circumvented by a process called translesion synthesis (TLS) in which DNA lesion is

bypassed by the Y-family DNA polymerases. PCNA monoubiquitination plays an important role in TLS genomic stability against mutation. However, regulation of PCNA monoubiquitination is not clear. We demonstrated in this study that ING1b enhanced PCNA monoubiquitination in a Rad18- and p53-dependent manner. Although ING1b is not required for Rad18 expression, it was required for PCNA and Rad18 foci colocalization. We also showed that histone hyperacetylation bypassed the requirement for ING1b in PCNA monoubiquitination. ING1b-enhanced PCNA monoubiquitination did not require interaction with PCNA but required the p300 histone acetyltransferase. Furthermore, ING1b is also important for cells to survive stalled replication forks. Cells lacking ING1b were defective in recovering from S phase blockage. There was also an increase in H2AX phosphorylation, a hallmark for double strand breaks, at S phase in ING1b knockdown cells after UV irradiation. These results indicate that ING1b is important in regulating PCNA monoubiquitination and stabilization of stalled replication fork after UV, linking chromatin remodeling and S phase recovery in genomic stability maintenance.

(9:18am)

SCREENING FOR MELANOMA AND NON-MELANOMA SKIN CANCER WITH ANNUAL CUTANEOUS EXAMINATION: REVIEW OF OUTCOMES & COMPLIANCE - PRESENTAION OF PRELIMINARY RESULTS

Shannon Humphrey MD, Katie Beleznay BSc, Jason Rivers MD FRCPC. Department of Dermatology and Skin Science, University of British Columbia, Vancouver, Canada

Justification: Annual cutaneous examination (ACE) is recommended by dermatologists as screening for melanoma and non-melanoma skin cancer (NMSC) in patients with risk factors for these cancers. However, there is no conclusive evidence to support this practice. Hypotheses: 1) Patients targeted for ACE have a higher incidence of skin cancer than patients not targeted for ACE. 2) Patients targeted for ACE have a higher incidence of skin cancer than the general population. 3) A significant percentage of patients targeted for ACE comply with this recommendation. Objectives: 1) To determine if patients targeted for and compliant with ACE develop melanoma or NMSC at a greater rate than those not targeted for ACE. 2) To determine if patients targeted for and compliant with ACE develop melanoma or NMSC at a greater rate than the general population. 3) To determine what percentage of patients comply with ACE. 4) To explore reasons for non-compliance with ACE. Methods: A retrospective review was completed using charts from a single dermatologist affiliated with the Department of Dermatology and Skin Science, UBC. A random sample of charts were selected from 1993-2001 using the BC Medical Services Plan diagnostic codes of 172: malignant melanoma of the skin (n=258), and 216: benign neoplasm of the skin (n=3956). The rates of melanoma and NMSC will be compared between the compliant ACE group and non-ACE group, and with the general population of BC. Patients targeted but non-compliant with ACE will be invited to participate in a survey that explores reasons for non-compliance.

(9:30am)

ZIPPER OR VELCRO - CADHERIN INTERACTIONS IN THE DESMOSOME

Gethin Rh. Owen [1,3] Devrim Acehan[2], KD Derr[1], William J. Rice[1], David L. Stokes [1,2.]
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Desmosomes are a complex assembly of protein molecules that form at the cell surface and mediate cell-cell adhesion. Desmosomes are particularly important in tissues that experience mechanical challenge, such as skin and heart where genetic or autoimmune defects are manifested in disease states. In addition to a structural role, recent reports have also indicated that, desmosomes can be dynamic and may play a role in cell-cell signaling. Much is known about the composition of desmosomes and there is an established consensus for the location of and interactions between constituent proteins within the assembly. Also, X-ray crystallography has determined atomic structures of isolated domains from several constituent proteins. Nevertheless, there is a lack of understanding about the architecture of the intact assembly and the physical principles behind adhesive strength of desmosomes therefore remain vague. We have used electron tomography to address this problem. In previous work, we investigated the in situ structure of desmosomes from newborn mouse skin preserved by freeze-substitution and imaged in resin-embedded thin sections. In the current work, we have isolated desmosomes from cow snout and imaged them in the frozen, unstained state. Although not definitive, the resulting images provide support for the irregular groupings of cadherin molecules previously seen in mouse skin.

(9:42am)

GENE EXPRESSION FEATURES OF LICHEN PLANOPILARIS AND PSEUDOPELADE OF BROCQ

Megan Isaac-Renton, Mei Yu, Elizabeth K. Ross, Magda Martinka, Jerry Shapiro, Kevin J. McElwee. Department of Dermatology and Skin Science, University of British Columbia, Vancouver, Canada

Pseudopelade of Brocq (PPB) and lichen planopilaris (LPP) are the most common primary cicatricial (scarring) alopecias encountered. Because these diagnoses share so many signs and symptoms, their distinct nosological identities have often been questioned. The purpose of this study was to provide empirical molecular evidence supporting the hypothesis that PPB and LPP are distinct diseases. Alopecia affected and clinically normal scalp tissue biopsies were obtained from 10 untreated patients with PPB or LPP. Microarray analysis, using a 21K expanded sequence verified cDNA set was performed. Differentially expressed genes were identified by Significance Analysis of Microarrays (SAM) evaluation and subsequently screened for signaling pathway involvement. Based on microarray screening results, 10 genes were selected for further study, based on their differential expression between samples diagnosed as PPB or LPP. DNA extracts from the scalp biopsies were examined using quantitative polymerase chain reaction (qPCR), a more sensitive method of mRNA expression quantification than microarray technology. Gene expression profiles in LPP and PPB versus normal scalp sclap biopsies were significantly distinct. The qPCR results from 3

of the 10 genes (MMP11, TNFSF13B, APOL2) showed distinct, verifiable gene expression profiles, while the results from the remaining 7 genes were inconsistent with the original microarray results. Further work is required to explore the reasons for these differences. The results indicate that PPB and LPP do in fact exhibit distinct gene expression profiles suggesting they have unique biological identities. This information may one day help to establish appropriate, and effective, treatment protocols for these diseases.

(11:00am)

EFFECT OF PHOSPHORYLATION ON THE PROTEASOMAL DEGRADATION OF THE TUMOR SUPPRESSOR P33^{ING1B}

Garate, M; Wong, R; Wang, Y and Li, G. Department of Dermatology and Skin Science, University of British Columbia, Vancouver, British Columbia, Canada.

The tumor suppressor p33^{ING1b} plays a prominent role in cellular stress responses including cell cycle arrest, apoptosis, chromatin remodeling, and DNA repair. However, its degradation pathway is still unknown. We have recently shown that genotoxic stress induces p33^{ING1b} phosphorylation at Ser126 and abolishment of Ser126 phosphorylation dramatically shortened its half-life. Based on these evidences, we hypothesize that S126 phosphorylation modulates the interaction of p33^{ING1b} with its degradation machinery, thus stabilizes this protein. By a strategy combining the use of inhibitors of the major degradation pathways of nucleus (proteasome and calpains), partial isolation of the proteasome complex and *in vitro* interaction and degradation assays we attempted to determine the degradation mechanism of p33^{ING1b}. We found that this protein is degraded in the 20S proteasome and NQO1, a quinone oxidoreductase previously shown to modulate the degradation of p53 in the 20S proteasome core, inhibits the degradation of p33^{ING1b}. Furthermore, UV irradiation induces phosphorylation at Ser126, which in turn facilitates p33^{ING1b} interaction with NQO1.

(11:12am)

***IN VIVO* CONFOCAL RAMAN SPECTROSCOPY FOR SKIN DISEASE DIAGNOSIS AND ASSESSMENT**

Hequn Wang, Harvey Lui, David I. McLean, Haishan Zeng. The Laboratory for Advanced Medical Photonics (LAMP), Department of Dermatology and Skin Science, University of British Columbia, and Cancer Imaging Department, BC Cancer Research Center, Vancouver, BC, Canada

Background: Confocal scanning laser microscopy (CSLM) is a superior technique which can image the tissue non-invasively in real-time and at cellular-level resolution. Raman Spectroscopy (RS) measures molecular vibrations and can provide fingerprint type specific signatures for molecular identification. We plan to combine CSLM with RS to non-invasively obtain depth-resolved biochemical information of *in vivo* skin and to perform chemical analysis of target skin microstructures. We believe this novel technology has great potential for improving skin diagnosis and for furthering our understanding of the relation between skin disease and biochemical changes in the skin. It can also be used to monitor the nature history of skin disease or biological process, study transdermal drug delivery, and monitor therapy responses. Objective: Design and

construct a novel instrument which would be used in clinical applications. It would be capable of performing skin morphology imaging at cellular-level resolution, and provide the capability of region of interest scanning to facilitate spectral measurements of targeted microstructures identified by the full field imaging. Methods: Step 1: Develop a RS system suitable for chemical analysis of skin microstructures *in vivo*. Step 2: Construct a CSLM system and integrate with the RS system. Step 3: Complete data acquisition, images reconstruction and processing and automatic control of the scanning unit. Step4: Build a probe practical for clinical measurements. Step 4: Test the developed instrument and guide system optimization. Step 5: Perform further clinical studies. In this presentation, current progress will be reported and future research plan will be outlined.

(11:24am)

CELL-MEDIATED CYTOTOXICITY IS AN UNLIKELY MECHANISM OF HAIR LOSS IN CHRONIC MOUSE ALOPECIA AREATA

Armin Barekatain, Jerry Shapiro, Kevin J McElwee. Department of Dermatology and Skin Science, University of British Columbia, Vancouver, Canada

Alopecia areata (AA) is a chronic inflammatory disease of hair follicles manifesting as patchy areas of hair loss on the scalp and body. Development of AA is associated with peri- and intra-follicular inflammation of anagen stage hair follicles, primarily by CD4+ and CD8+ cells. To study whether cell-mediated cytotoxicity against the follicular epithelium is a component of the hair loss disease mechanism, we examined gene and product expression profiles typical of cytotoxic cells in the C3H/HeJ mouse model for AA, using quantitative reverse transcriptase PCR (RT-PCR) and immunohistochemistry. mRNA expression of FasL, Granzyme A, Granzyme B, pro- and anti-inflammatory cytokines were highly up-regulated in the skin of AA-affected mice. Immuno-histochemical studies of the skin revealed that, although greater numbers of granzyme B and FasL expressing cells were present in AA affected skin, the cells were morphologically diffusely distributed and not exclusively located within the focal peri- and intra-follicular infiltrate. The few identified Perforin expressing cells also failed to show any association with the follicular infiltrate in AA affected mouse skin and TUNEL staining suggested relatively limited apoptosis activity in the hair follicle epithelium. While Granzymes and FasL may play important roles in disease development, the profiles and patterns of expression are not consistent with direct cell-mediated cytotoxic action against the follicular epithelium in chronic mouse AA. Potentially, hair growth inhibiting cytokines may play a more dominant role in AA development than previously thought.

(11:36am)

AURORA LIGHT AND WHITE HAIR REMOVAL EFFICACY AS EXPERIENCED BY THE LASER AND LIGHT HAIR REMOVAL CLINIC

Nina MacDonald, RN, BScN, DNC (1), Nina Otberg, MD (2), Jerry Shapiro, MD, FRCPC (1,2), Harvey Lui, MD, FRCPC (1,2), Vincent Ho, MD, FRCPC (1,2), David Zloty, MD, FRCPC (1,2), Larry Warshawski, MD, FRCPC (1,2). 1 Skin Care Centre, 2 Department of Dermatology & Skin Science, University of British Columbia, Vancouver, Canada

While literature documents the effectiveness of the Aurora (IPL coupled with Radio Frequency) in the removal of white and light hair, it was felt by the Skin Care Centre nurses that the results were less than optimal. We are proposing to do a chart review on patients seen and treated at the Laser and Light Hair Removal Clinic of the Skin Care Centre. Their responses to treatment will be quantified and variables that potentially affect the efficacy of treatment will be identified and put on a graph for comparison. Our findings will be analyzed and compared with those from literature. Settings used, patient selection criteria and other variables will be compared with those cited in the literature and guidelines provided by the manufacturer. Potentially, depending on our findings and hypothesis, further research may be considered looking at different treatment settings, our ability to replicate published study findings and a more accurate way of measuring outcome. This is an area of hair removal where patients' needs are currently unmet. Variables that are being considered are: poor selection of client, less than optimal treatment parameters, pre-existing medical conditions and the accuracy of the method of measuring improvement.

(11:48am)

DEVELOPMENT AND APPLICATION OF A NON-REJECTABLE SKIN ALLOGRAFT USING K14-DRIVEN IDO TRANSGENIC MOUSE SKIN

Darya Habibi[1, 2], Reza B. Jalili[1], Jim Peacock[2], Dieter Fink[2], Rupi Dhesi[2], Anita Gertz[2], 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: Skin transplantation is one way of coping with non-healing wounds. Indoleamine 2, 3-dioxygenase (IDO), is a strong immunomodulatory enzyme that breaks down tryptophan. We aim to develop a novel method to prevent skin graft rejection. Induction of IDO in the allograft creates a tryptophan deficient microenvironment in which infiltrated immune cells, but not allogeneic skin cells, are unable to survive, proliferate and destroy the engrafted skin. For this, we develop a strain of K14-driven IDO transgenic mice. K14 promoter targets the transgene expression to basal keratinocytes. At this stage, we attempt to develop ubiquitin (general)-driven IDO transgenic mice to achieve the systemic induction of IDO. Materials and Methods: K14-driven IDO lentiviral vector will be constructed and IDO expressing lentivirus will be made and used to infect mice embryos to develop transgenics. IDO transgenic mouse skin will be used as allograft. Graft recipient animals will be followed up to evaluate the graft survival. Results: We have developed and validated the ubiquitin driven IDO expressing lentiviral vector as well as its lentivirus. We used the lentivirus to develop ubiquitin-driven IDO transgenic mice. Embryos developed well up to the blastocyst stage. After several trials of embryo implantation, no pups were born. Developing the transgenic mice for the mock virus was successful. We have constructed the K14-driven IDO expressing lentiviral vector. Conclusion: Systemic over-expression of IDO might be lethal during embryogenesis. To achieve the restricted expression of IDO to the keratinocytes, we will develop the tissue specific K14- driven IDO transgenic mice.

(12:00noon)

IMMUNOMODULATORY EFFECTS OF TOPICAL CALCIPOTRIOL ON SUBCUTANEOUS VACCINATION.

Bach PJ, Ghoreishi M and JP Dutz. Department of Dermatology, University of British Columbia, Vancouver, BC, Canada.

1,25-dihydroxyvitamin D3 (Vitamin D) is a potent immunomodulator capable of generating regulatory T cells (Tregs) *in vitro* as well as in murine models. Additionally, Vitamin D has been shown to promote mucosal immunity when used as a vaccine adjuvant. The synthetic Vitamin D analog calcipotriol used in conjunction with a transcutaneous immunization protocol is capable of inducing antigen-specific Tregs. We show here that pretreatment of an area of skin with calcipotriol also has significant effects on the immune response to subcutaneously injected vaccines. Functionally, calcipotriol treatment results in minor suppression of the proliferation of CD4+ T cells while markedly inhibiting CD8+ T cell priming in response to subcutaneous vaccination, despite the topical co-administration of a potent TH1 inducing TLR9 agonist. Immunization under a pretreated area is also capable of inducing a population of CD4+CD25+ Tregs. The humoral response to vaccination is affected by calcipotriol treatment as well, evidenced by a marginally increased production of IgG1 immunoglobulins along with a strong suppression of the IgG2a isotype. This is in contrast to the corticosteroid beta-methasone, which also inhibits CD8+ T cell priming but strongly suppresses the production of both IgA and IgG1 antibodies. Our data indicates that calcipotriol has distinct effects on immune responses to subcutaneous vaccines and that further research is warranted into its potential as a topical immunomodulator. (Research funded by CIHR grant no. 160744)

(12:12pm)

NASAL SUBUNITS AND MOHS: OPTIMIZING RESECTION TECHNIQUE BY UTILIZING RECONSTRUCTIVE PRINCIPLES

Justin H. Piasecki, Bryce J. Cowan. Department of Dermatology and Skin Science, University of British Columbia

In the interest of ensuring optimal resection, classic surgical oncologic teaching regarding the treatment of non-melanotic skin cancer maintains that tumor extirpation should be approached singularly, without regard for future reconstruction. The subunit principle of nasal reconstruction holds that the finest cosmetic result following reconstruction of defects involving greater than or equal to 50% of an aesthetic subunit is best achieved if the remaining tissue within that subunit is resected and the entire subunit reconstructed as a whole. We argue that combining these two principles for Mohs surgery of nasal cancers can contribute positively to both resection and reconstruction results. Specifically, if during micrographic excision of nasal tumors, the surgeon estimates that the next level of excision will result in a defect greater than or equal to 50% of an aesthetic subunit, the next Mohs level should include the entirety of that subunit. In this way, time is saved during extirpation, potentially decreasing the number of required levels to reach cure. Likewise, the defect created can then be reconstructed as an entire subunit, optimizing aesthetic outcome. 10 consecutive patients with non-melanotic nasal skin cancers with positive first Mohs levels were

treated with this approach. Patient satisfaction and cosmetic outcomes are currently being measured as final reconstructive stages are concluded.

(1:30pm)

USING TOPICAL TLR AGONISTS TO IMPROVE VACCINE RESPONSES: TLR9 AGONISTS INDUCE HOMING RECEPTOR EXPRESSION IN RESPONDING CYTOTOXIC T LYMPHOCYTES

Cheng, Wing-ki (Vicki) and Dutz, J.P. Child & Family Research Institute, Department of Dermatology & Skin Science, and University of British Columbia

Current vaccine technologies remain inadequate to counter the risk of pandemics of viral pathogens. This is in part due to the poor ability to induce effective antiviral immunity mediated by cytotoxic T lymphocytes (CTLs). Our group previously demonstrated that topical administration of immunostimulatory CpG oligodeoxynucleotide (ODN), a TLR9 agonist, induced more potent, rapid and durable antigen (Agn)-specific CTL responses to locally injected protein Agn. We proposed that topical TLR9 family agonists can safely optimize vaccine immunogens using skin as an immunization route. To confirm these results, we performed *in vivo* CFSE proliferation assay to determine the ability of CpG adjuvant to enhance the priming of Agn-specific CTLs. C57BL6 mice were adoptively transferred with large numbers (3-5 x 10^6) of CFSE-labeled OT-1 CD8 cells and immunized by subcutaneous injection with ovalbumin (OVA) protein as model Agn. In contrast to previous results with un-manipulated mice, immunization with OVA with or without CpG adjuvant induced the proliferation of these cells as determined by CFSE dilution. Preliminary data suggested that OVA-specific CTLs generated with or without CpG adjuvant possessed different phenotypes. Agn-specific CTLs generated in the presence of CpG adjuvant demonstrated higher levels of P- and E-selectin ligand expression than those generated from immunization with subcutaneous OVA injection alone. These findings suggest that TLR activation can be used to modulate the homing characteristics of CTLs. Thus, TLR activation at the time of immunization through the skin may improve protection against subsequent infectious agent challenge by regulating the homing characteristics of the Agn-specific CTLs.

(1:42pm)

CALCULATION OF DONOR HAIR DENSITY, STRIP SIZE AND TRANSECTION RATES IN HAIR RESTORATION SURGERY

Nina Otberg, Andreas Mario Finner, Wen-Yu Wu, Hoon Kang, Abdullateef Alzolibani, Kevin McElwee, Luciana Zanet, David Zloty, Larry Warshawski, Jerry Shapiro. Department of Dermatology and Skin Science, University of British Columbia, Vancouver

Hair restoration surgery is an important treatment option in pattern hair loss. Exact calculation of the donor area and the expected number of transplanted hair follicles is crucial for patient satisfaction and efficient cost calculation. The aim of this study is to provide data on donor area hair density in hair transplantation before the procedure, using macro-photography and digital imaging software. Hair density will be compared to the number of extracted hair per cm². Digital macro-photographs of 20 fold magnification are taken before and after administration of tumescent anesthesia directly before the donor strip harvesting. Hair density will be measured using the digital imaging tool and

software Trichoscan®. The total expected number of follicles in the donor strip is calculated and later correlated to the number of harvested and transplanted hair. 27 male and female patients were enrolled in the study. Hair density ranged between 110 and 192 terminal hair/cm^2. After the administration of the local anesthetic the skin stretches and hair density decreased by 1-10%. The number of the harvested and transplanted hairs correlated well with the calculated number. The transection rate was less than 4%. Trichoscan® technique allows a better calculation of the donor strip size and dissection and transection rates. Transection and dissection rate can be calculated more exactly. Decrease in hair density of up to 10% after the administration of the tumescent anesthesia has to be taken into account.

(1:54pm)

ACCELERATED CUTANEOUS WOUND HEALING IN BETA6 INTEGRIN-DEFICIENT MICE IMPAIRED BY DEXAMETHASONE

Yanshuang Xie[1], Kai Gao[2], Lari Häkkinen[1], and Hannu Larjava[1]
[1]Faculty of Dentistry, The University of British Columbia, Vancouver, Canada;
[2]School of Dentistry, The University of California, Los Angeles, USA

Integrin $\alpha v\beta 6$ is an epithelial-specific receptor that is absent from healthy epidermis but de novo synthesized in wound repair. Studies have indicated that $\alpha v\beta 6$ integrin binds and activates latent TGF-β1. Integrin-mediated TGF-β1 activation has been shown to be the main activation mechanism of TGF-β1 in vivo. $\alpha v\beta 6$ integrin-dependent activation of TGF-β1 has also been confirmed to be pivotal in mouse models of multiple epithelial organs including lung, kidney and liver, suggesting that this mechanism may be of general importance. Both positive and negative effects of TGF-β1 on wound healing have been reported. However, the underlying mechanisms are largely unknown. As a potent endogenous activator of TGF-β1, the function of $\alpha v\beta 6$ integrin in wound healing remains to be determined. In the present study, impaired wound model induced by dexamethasone was established in $\beta 6$ integrin-deficient ($\beta 6$-/-) and wild-type (WT) mice. The results showed that wound healing was significantly accelerated in dexamethasone-treated $\beta 6$-/- mice compared with corresponding WT mice, characterized by an increased rate of granulation tissue formation, re-epithelialization and basement membrane regeneration. Dexamethasone-treated $\beta 6$-/- mice showed enhanced keratinocyte proliferation in both wound epithelium and hair follicles while the production of pro-inflammatory cytokines and TGF-β1 was reduced. Our data suggest that accelerated wound repair in dexamethasone-treated $\beta 6$-/- mice is associated with depressed anti-proliferative and pro-inflammatory effects of TGF-β1. Delayed wound repair that occurs in dexamethasone-impaired mice can be at least partially reversed by suppressing $\alpha v\beta 6$ integrin expression in skin, which may provide a specific target for future therapeutic intervention in impaired wound healing.

(2:06pm)

NUCLEAR FACTOR KAPPA B SUBUNIT P50 PROMOTES MELANOMA ANGIOGENESIS THROUGH UPREGULATING INTERLEUKIN-6 EXPRESSION

Alison Karst, Kai Gao, David Ngan, Jun Li, Ronald PC Wong, 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 NF-κB transcription factor subunit p50 is overexpressed in several tumor types, including melanoma. However, the significance of NF-κB p50 in tumor pathogenesis remains unclear. We used cDNA microarray to characterize the gene expression profile of melanoma cells overexpressing NF-κB p50 and found that the cytokine *IL-6* was highly upregulated in these cells. Elevated *IL-6* expression was confirmed in separate experiments using real-time PCR and ELISA. As *IL-6* is a potential pro-angiogenic factor, we hypothesized that NF-κB p50 may promote melanoma angiogenesis through *IL-6* upregulation. Using an *in vitro* human umbilical vein endothelial cell (HUVEC) proliferation assay and *in vivo* matrigel plug assay, we show that NF-κB p50-expressing melanoma cells enhanced the growth of endothelial cells and promoted blood vessel formation. Furthermore, silencing of NF-κB p50 or expression of its negative regulator, ATF3, abrogated the effects of NF-κB p50-mediated *IL-6* upregulation. Our data suggest that NF-κB p50 plays an important role in promoting melanoma angiogenesis by inducing *IL-6* expression and thus enhancing endothelial cell growth.

(2:18pm)

PERCEPTION OF TEXTURE IN SKIN LESIONS

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

This study aims to improve systems that automatically diagnose melanoma by introducing a reliable quantification of texture in skin lesions. These systems take as input an image of a skin lesion under high magnification and extract features from the image known to predict malignancy (such as: size, shape, colour, etc.). Statistical methods are then used to discriminate benign and malignant lesions based on these extracted features. It is known that textural information plays a large role in dermatologists' ability to diagnose melanoma. This is apparent when examining Menzies' scoring method or the 7-point checklist; terms such as 'atypical pigment network', 'radial streaming', etc. define specific textural patterns that are predictive of melanoma. Such descriptions though lack an explicit definition which makes it difficult for automated methods to identify them accurately. Additionally, most mathematical models of texture operate on a single channel grayscale image, rather than a 3-channel colorspace. A perceptual experiment was designed and conducted to determine: 1) If textural information alone can predict atypia and 2) Does the presence/absence of colour affect accuracy. The experiment was conducted on 5 subjects, and the results were analyzed using the Dorfman-Berbaum-Metz (DMB) method of receiver operating characteristic (ROC) analysis as well as a two one-sided test (TOST) of equivalence.

Results indicate that textural information alone can predict atypia and that the perception of this textural information is independent of colour.

(2:30pm)

REGULATION OF ING3 DEGRADATION BY PROTEASOME-UBIQUITIN PATHWAY

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The novel tumor suppressor ING3 has been shown to modulate transcription, cell cycle control, and apoptosis. In melanoma cells, we have reported that ING3 promotes UV-induced apoptosis via the Fas/caspase-8 dependent pathway. We also demonstrate that nuclear ING3 expression was remarkably reduced in malignant melanomas, and the reduced nuclear ING3 expression was significantly correlated with a poorer disease-specific 5-year survival of patients with primary melanoma. In melanoma cells, over-expression of ING3 significantly reduced colony formation, inhibited cell growth and induced cell cycle arrest at G1 phase. To investigate the regulatory mechanism of cellular ING3 level, we characterize the turnover pathway of ING3 protein in the current study. Our data revealed that ING3 protein was stabilized in the presence of proteasome inhibitor MG132. The ING3 protein was ubiquitinated and the ubiquitination level was increased in the presence of MG132, suggesting that ING3 protein is degraded in ubiquitin-dependent proteasome pathway. Moreover, the protein sequence between amino acid 96 and 111 is required for the ING3 degradation through proteasome pathway. Further study indicated that lysine residue K96R mutant completely abrogated the ubiquitination and degradation of ING3 protein. We also find that overexpression of Cullin1, a key component of SCF (Skp1/Cullin1/Roc1/F-box proteins) E3 ligase complex, significantly reduced ING3 expression and ING3 was physically interacted with both Cullin1 and Roc1. In addition, Cullin1 enhanced the ubiquitination and degradation of ING3. Taken together, our present data indicate that ING3 protein is degraded through SCF E3 ligase-mediated ubiquitin-proteasome pathway, which requires the ubiquitination of ING3 at K96 site.

(2:42pm)

MICRO-RAMAN IMAGING OF NORMAL AND MALIGNANT HUMAN SKIN CELLS

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Understanding the molecular changes that occur in cells is an important aspect in the multi-dimensional initiative to diagnose, treat, and ultimately cure cancers. Several methods are available for obtaining molecular information at a cellular level, but many of these methods involve introducing exogenous agents, or can only be applied to *ex-vivo* or fixed cells. Micro-Raman spectroscopy has the potential to overcome these obstacles; it involves shining high intensity laser light on the sample and measuring the scattered light. As an initial trial, we used the method to obtain molecular images of fixed

keratinocytes, melanocytes and malignant cells of the human skin. The cells were either located within thin sections of skin biopsies or on glass slides seeded with cultivated cells. Autofluorescence emission from tissue is generally very intense which makes measuring the much weaker Raman emission difficult; however in the cells we used the fluorescence was surprisingly low. Excitation power at the sample was kept to approximately 6 mW to avoid damaging the cells; this was the limiting factor on how quickly a Raman image could be obtained. Despite this difficulty we were able to obtain Raman images with rich information about the spectroscopic and structural features within the cytoplasm and cell nuclei. Differences were observed between the Raman images of normal and malignant cells. In the talk we shall explain the technique, the experimental setup, and show some of the images we obtained.

(2:54pm)

DEMOGRAPHIC AND TUMOUR CHARACTERISTIC CHANGES OF PATIENTS DIAGNOSED WITH NONMELANOMA SKIN CANCER FROM 1993 TO 2005 IN VANCOUVER, BRITISH COLUMBIA

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BACKGROUND: Nonmelanoma skin cancer (NMSC) including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most common malignancies diagnosed in Caucasian populations. The incidence of BCC and SCC is increasing worldwide, however this varies by region. To date, there is limited data about the trends of NMSC in Canada. OBJECTIVE: To determine the demographic and tumour characteristic changes of patients diagnosed with BCC and SCC from 1993 to 2005 in a dermatology practice in Vancouver, British Columbia. METHOD: A retrospective chart review was conducted on patients with biopsy confirmed NMSC between 1993 and 2005. Demographic and tumour characteristics were documented for the first two incident BCCs and SCCs per patient and a descriptive data analysis was undertaken. RESULTS: 1177 NMSC were identified from 885 patient charts. The number of BCCs increased from 1993 to 2003 then decrease until 2005. Temporal trends for SCC were difficult to discern due to low counts. BCCs and SCCs were generally diagnosed in an older age group (60+), however an important group of younger patients (20-39 yrs) was diagnosed with BCCs. BCCs and SCCs were most commonly seen on the Head and Neck. However, the leg was a common location for SCC in women. CONCLUSION: NMSC is prevalent in British Columbia. These results highlight the fact that NMSC can affect individuals younger than 40 years old. Prevention strategies are still warranted to reduce the burden of NMSC in British Columbia.