

## ORAL PRESENTATIONS

(8:30am)

**CHK1 AND CDK1 INFLUENCE THE TURNOVER RATE OF P33ING1B VIA PROTEIN PHOSPHORYLATION.** Eric I. Campos<sup>1</sup>, Jason A. Bush<sup>2</sup>, Marco Garate<sup>1</sup>, Wei-Hung Kuo<sup>1</sup>, Tristan A. Williams<sup>2</sup>, Hao Xiao<sup>3</sup>, Jeffrey W. Smith<sup>2</sup> and Gang Li<sup>1</sup>. <sup>1</sup>Department of Medicine, University of British Columbia, Vancouver, British Columbia V6G 3Z6, Canada; <sup>2</sup>The Burnham Institute, La Jolla, California 92037, USA; <sup>3</sup>ImmuneChem Pharmaceuticals Inc., Burnaby, British Columbia V5J 3M6, Canada.

ING (Inhibitor of Growth) proteins are evolutionary conserved co-factors of histone acetyltransferases and histone deacetylases that mediate the acetylation of essential cellular components including histones and the p53 tumor suppressor, thus modulate cell-cycle checkpoints, apoptosis and ultimately tumor suppression. Among the ING family members, p33ING1b, the most intensively studied member, plays an important role in the cellular stress response to DNA damage. Through a newly described methyl esterification method that allows easier detection of negatively charged residues during MALDI-TOF mass spectrometry, we were able to demonstrate that p33ING1b is phosphorylated at serine 126 in response to DNA damage. The mutation of serine 126 to alanine dramatically shortened the half-life of p33ING1b. Through kinase profiling, we found that both Chk1 and Cdk1 are able to phosphorylate this residue. However, while Cdk1 is responsible for basal phosphorylation of p33ING1b serine 126 in the absence of DNA damage, Chk1 predominantly phosphorylates this residue upon genotoxic stress. ATM directed RNAi reduced phosphorylation of serine 126 in the presence of DNA damage but ATM does not phosphorylate this residue. These data indicate that p33ING1b is a component of the ATM/ATR response cascade to genotoxic stress.

(8:42am)

**ING FAMILY MEMBERS PROMOTE CHROMATIN RELAXATION FOR NUCLEOTIDE EXCISION REPAIR OF UV-INDUCED DNA LESIONS.** Wei Hung Kuo, Eric I. Campos, Gang Li. University of British Columbia, Vancouver, BC, Canada.

Eukaryotic DNA is packaged into a higher order structure called chromatin which consists histones and other chromatin-associated proteins. This tight intermolecular structure regulates a variety of processes such as mitosis, transcription and DNA repair by restraining access of cellular machineries to DNA. Numerous multi-protein complexes, such as histone acetyltransferases (HAT) that transfer an acetyl moiety to the N-terminal lysine residues within the histone tails can modify chromatin to confer its flexibility. The mammalian ING1 (inhibitor of growth 1) gene encodes a protein that has been implicated in apoptosis, senescence, cell cycle checkpoint control, transcriptional regulation and DNA repair. ING family proteins (ING1-5) associate with a spectrum of HAT and histone deacetylase (HDAC) complexes that target various substrates including the p53 tumor suppressor and histones. We have demonstrated that ING1b enhances the repair of ultraviolet (UV) light-damaged DNA. We hypothesize that the

ING family members enhance DNA repair by promoting a global chromatin relaxation through histone acetylation. We showed that in an immunofluorescent study that ING1b mobilizes globally within the nucleus upon localized UV-light infliction. Micrococcal nuclease digestion of inter-nucleosomal DNA confirmed that ING1b could induce a global chromatin relaxation after UV-induced genomic injury. We also show that all the ING family members can enhance DNA repair in different cell lines. Therefore, we proposed that ING family members associate with HAT activity to alleviate chromatin condensation for DNA repair. Elucidating these mechanisms will contribute to the knowledge of cellular maintenance of genomic integrity.

**(8:54am)**

**EMOTIONAL AND PSYCHOLOGICAL IMPACT OF MOHS MICROGRAPHIC SURGERY ON FEMALE PATIENTS.** Robert Hayes, MD, FRCPC, Dermatologic Surgery Fellow, UBC Dermatology. Bryce J. Cowan, MD, PhD, FRCSC. David M. Zloty, MD, FRCPC.

**Introduction:** Mohs Micrographic Surgery (MMS) is a tissue-sparing technique that offers exceptionally high cure rates for difficult skin cancers on the head and neck. Although the Mohs surgeon's goal is complete tumor clearance as well as an excellent aesthetic outcome after reconstruction, MMS can be a stressful experience for patients. The goal of the proposed study is to assess the emotional and psychological impact of MMS on our female patients, to identify ways to minimize this trauma, and find ways to better prepare our patients for MMS. **Methods:** Prospective interviews and questionnaires assessing self-image and anxiety will be performed on all female patients undergoing MMS at three time points: prior to MMS, after the surgical reconstruction is completed, and at three-month follow-up. **Results and Conclusions:** Preliminary results will be discussed with the audience.

**(9:06am)**

**IDENTIFICATION OF THE DEGRADATION PATHWAY OF THE TUMOR SUPPRESSOR P33<sup>ING1B</sup>.** Marco Garate, Eric Campos, and Gang Li. Department of Medicine, Division of Dermatology, University of British Columbia, Vancouver, British Columbia, Canada.

The tumor suppressor p33<sup>ING1b</sup> plays a prominent role in cellular stress events including cell cycle arrest, apoptosis, chromatin remodeling, and DNA repair. We have previously shown that in melanoma cell lines ultraviolet irradiation induced p33<sup>ING1b</sup> expression in a time- and dose-dependent manner. Recently, we have shown that drug-treatment or events that evoke DNA-damage in melanoma cells induce a rapid phosphorylation of p33<sup>ING1b</sup> and for the first time we identified the Ser126 residue to be phosphorylated. Noteworthy, abolishment of Ser126 phosphorylation dramatically shortened the half-life of p33<sup>ING1b</sup>, suggesting that Ser126 phosphorylation may play an important role in the biological functions of this protein. Despite these advances in biochemical characterization of p33<sup>ING1b</sup>, the degradation pathway of this tumor suppressor is still unknown. Based in these evidences, we hypothesize that phosphorylation of the Ser126 residue blocks the interaction of p33<sup>ING1b</sup> with its degradation machinery, thus stabilizes this protein. Treating melanoma cells with inhibitors of the major

degradation pathways of nucleus (proteasome and calpains) and cytoplasm (lysosomes) we have attempted to determine the degradation mechanism of p33<sup>ING1b</sup>. The specific inhibitor of lysosomal function (ammonium chloride) inhibited the degradation of p33<sup>ING1b</sup> suggesting that lysosomes are at least part of the degradation pathway of this tumor suppressor. Since the expression of a protein is a balance of synthesis and degradation, the characterization of the degradation mechanism of p33<sup>ING1b</sup> would help to understand the fine expression changes induced by cell stress and would serve as a model to investigate the degradation pathway of more essential tumor suppressors.

**(10:10am)**

**COLLAGEN TRIPLE HELIX REPEAT CONTAINING (CTHRC1), A NOVEL CANCER ASSOCIATED PROTEIN, IS INCREASED IN ADVANCED MELANOMAS AND PROMOTES CANCER CELL MIGRATION AND SURVIVAL.** Liren Tang,<sup>1,2</sup> Mingwan Su,<sup>1,2</sup> Derek Dai,<sup>1</sup> Magdalena Martinka,<sup>3</sup> Yi Zhang,<sup>1,2</sup> Gang Li<sup>1</sup> and Youwen Zhou<sup>1,2</sup>. <sup>1</sup>Division of Dermatology, Department of Medicine, and <sup>2</sup>Chieng Genomics Centre, Laboratory of Predictive Medicine and Therapeutics, Vancouver Coastal Health Research Institute, and British Columbia Cancer Agency, <sup>3</sup>Department of Pathology, University of British Columbia, Vancouver, Canada.

The CTHRC1 (collagen triple helix repeat containing 1) is a pro-migratory protein first found during rat tissue repair process. Accumulating evidences support that tissue repair and carcinogenesis are tightly linked. However, no investigation has addressed on the roles of CTHRC1 gene in human carcinogenesis. This study was designed to characterize the clinical and functional relevance of CTHRC1 in melanoma and other human solid cancers. First, semi-quantitative immunohistochemistry analysis was performed on 304 clinically-annotated, paraffin-embedded biopsies representing different stages of melanoma progression. In benign nevi and non-invasive melanoma biopsies, there was little CTHRC1 protein expression. In contrast, in invasive primary melanomas, a significant increase of CTHRC1 protein was observed. There was a further increase of CTHRC1 protein in metastatic melanoma specimens compared with non-metastatic lesions. Second, the CTHRC1 mRNA expression was surveyed in 310 samples representing 19 types of human solid cancers. The aberrant CTHRC1 transcription was found in 16 cancer types, especially cancers of the gastrointestinal tract, lung, breast, thyroid, ovarian, cervix, liver and the pancreas. Finally, when the expression of CTHRC1 protein was inhibited by short interfering RNA on cultured melanoma cells, cell migration and cell survival were significantly reduced. We conclude that aberrant expression of CTHRC1 is widely present in human solid cancers, and appears to be associated with cancer tissue invasion and metastasis in melanoma. This novel cancer-associated gene may play important functional roles in tumor progression by promoting both cell survival and migration. Therefore, targeting CTHRC1 gene may represent an attractive approach for developing cancer therapies.

(10:22am)

**INHIBITION OF CTHRC1 INCREASES TEMOZOLOMIDE CHEMOSENSITIVITY OF CULTURED MELANOMA CELLS.** Wency Ip, Liren Tang, Mingwan Su, and Youwen Zhou. Chieng Genomics Center, Laboratory of Predictive Medicine and Therapeutics, Vancouver Acute Health Research Institute, and Division of Dermatology, Department of Medicine, University of British Columbia.

**Background:** Melanoma is the most threatening form of skin cancer due to its rapid increase in incidence and its high resistance to chemotherapy drugs such as dacarbazine (DTIC) or its derivative temozolomide (TMZ). Previous studies in our laboratory focused on identifying molecular targets with therapeutic potentials. Recently, it has been discovered that collagen triple helix repeat containing 1 (CTHRC1) is highly expressed in metastatic melanoma cells and not in nevi. Further investigations into CTHRC1 have shown that this gene is involved in melanoma cell survival. Hence, we attempt to find out if the chemosensitivity of melanoma cells can be enhanced by inhibiting CTHRC1 gene expression. **Methods:** Two melanoma cell lines, KZ-28 and MMRU, which have high expression of CTHRC1, were used in this study. Two different CTHRC1 small interference RNA (siRNA) constructs were transfected into the cell lines using lipofectamine. Six hours after transfection, different concentrations of TMZ ranging from 50 - 1000  $\mu$ M were added to the cells. Cell survival was later assessed by the MTT assay.

**Results:** For both cell lines, both CTHRC1 siRNA constructs were able to reduce cell survival after 3 days in the absence of TMZ. With the addition of TMZ at low concentrations, the differences in cell survival were even greater. However, at high TMZ concentration the differences in cell survival were reduced, especially in MMRU cell lines because most of the cells were dead regardless of their treatment. **Conclusion:** Chemosensitivity of metastatic melanoma cells can be enhanced by inhibiting CTHRC1 gene expression.

(10:34am)

**MICROARRAY ANALYSIS OF GENE EXPRESSION IN BASAL CELL CARCINOMAS.** Mei Yu, David Zloty, Laurence Warshawski, Bryce Cowan, Nicholas Carr, Jerry Shapiro, Kevin J McElwee. Division of Dermatology, Division of plastic surgery, Department of medicine, University of British Columbia.

**Background:** Basal cell carcinoma (BCC) is the most common form of human cancer. Though the aberrant signaling role of the Hedgehog pathway is understood, little is known about the downstream genetic mechanisms underlying basal cell carcinomas. BCC subtype has been based on clinical and histology patterns, but remains inexact. The characterization of molecular events would improve our understanding of carcinogenesis and classification. **Objective:** To identify differentially expressed genes in different morphological types of BCC as compared to normal skin epithelium. To search for genes associated with tumorigenesis promotion and to define products significant in BCC survival and growth. **Method:** Microarray analysis, using an expanded sequence verified set of Human Genome 21K cDNA glass array, was performed on tissues obtained from previously untreated patients undergoing surgical resection (6 superficial,

6 nodular, 6 morphea form, 7 normal skin). The significantly differentially expressed genes were identified using analysis of microarray results in various data sets. Selected genes were validated using real-time PCR analysis using an expanded set of 27 BCC samples. **Result:** The global gene expression profiles in different BCC subtypes and normal skin were distinguishable by unpaired T test and one –way ANOVA-analyses. 88 genes were at least 5 fold differentially expressed between BCC (all morphological types) and normal skin ( $p < 0.01$ ). Many of these differentially expressed genes were involved in Hedgehog, the cell cycle, apoptosis, angiogenesis, calcium signaling and other signal transduction regulatory pathways. Other categories of genes showing statistics differential expression between different subtypes were associated with cytoskeleton, extracellular matrix, and cell metabolism pathway. **Conclusion:** Our findings may have important implications for understanding the pathogenesis of BCC.

**(10:46am)**

**CLINICAL STUDIES IN HAIR TRANSPLANTATION.** Finner A, McElwee K, Zloty D, Warshawski L, Rivers J, Hoffmann R, Shapiro J. Clinical Postgraduate Trainee in Hair, UBC Division of Dermatology.

**Background:** Hair transplantation (HT) has become an important surgical treatment option in androgenetic alopecia with advancements in technique such as strip harvesting and follicular unit grafting. But quantitative evidence based on data on regrowth is still lacking. This is partly due to general difficulties when assessing hair growth. Unique problems in this setting include variations in technique, inadequate sample size, incomplete follow-ups and a lack of reproducible methods. **Methods:** Trichoscan® (TS) is a new tool to measure hair growth parameters. For repeated measurements, the target area is marked by a pin-point temporary tattoo or an angioma is used as a landmark. Two studies have been initiated at the UBC transplant centre after obtaining ethics board approval. Study 1 will observe hair density in a target area before and after HT. The number of hairs transplanted into the target area is counted. TS is performed before the procedure and after 2 weeks, 4 and 6 months. In the subsequent study, occipital donor hair density is assessed with TS before and after tumescent anesthesia prior to strip removal and compared to the actually harvested hairs per cm<sup>2</sup>. **Results:** Practicability of TS and patient feedback will be discussed. Initial results from study 1 show post-op shedding and thicker hairs on follow-up visits. **Discussion:** TS appears to be a promising, objective tool to assess hair growth. From these and similar studies, we expect new insights into the dynamics of hair growth following HT.

(10:58am)

**EPICUTANEOUS APPLICATION OF THE VITAMIN D ANALOGUE CALCIPOTRIOL TO INDUCE SYSTEMIC TOLERANCE.** Mehran Ghoreishi, Jennifer Obst and Jan P Dutz. Dept of Medicine and Research Institute for Child and Family's health, University of British Columbia, Room 218, 950 West 28th Avenue, Vancouver, BC.

In a murine model,  $1\alpha, 25$ -Dihydroxyvitamin D<sub>3</sub> (Vitamin D) induces tolerance *in vitro* and *in vivo* when administered orally or intraperitoneally in combination with other immune suppressive drugs. This study investigates whether topical application of a vitamin D analogue (calcipotriol) affects subsequent T cell responses to ovalbumin (OVA) immunization. Mice were treated with calcipotriol on their back skin and then were OVA immunized transcutaneously (transcutaneous immunization – TCI). Calcipotriol prior to TCI induced tolerance or suppression of subsequent antigen-specific CD8<sup>+</sup> T cell proliferation and IFN $\gamma$  production in C57Bl/6 mice. This effect was antigen dependent and mediated by CD4<sup>+</sup>/CD25<sup>+</sup> T regulatory cells (T<sub>reg</sub> cells) expressing high levels of FoxP<sub>3</sub>, since transfer of these cells from vitamin D treated and OVA immunized but not irrelevant (BSA) immunized mice resulted in suppression of OVA-specific CTL responses in naïve recipients. F4/80 is a molecule expressed by tolerance inducing macrophages. In our study F4/80<sup>+</sup> cells were increased in draining lymph nodes of mice treated with calcipotriol. To determine if tolerance-inducing effect of calcipotriol can prevent an immune response in organs other than skin, pathological evidence of OVA induced allergic asthma and effect of calcipotriol-OVA vaccination on this was studied. Histological examination of mice immunized with OVA followed by OVA immunization onto calcipotriol treated skin showed diminished peri-bronchoalveolar cell infiltration induced after intranasal OVA inhalation when compared to mice not treated with calcipotriol prior to TCI. These studies thus describe a new method for systemic antigen specific tolerance induction.

(11:10am)

**THE ROLE OF INTERFERON- $\alpha$ , ULTRAVIOLET B LIGHT AND CELL DEATH IN AUTOIMMUNITY.** Aaron Wong, Mehran Ghoreishi, MD, PhD, Jan P Dutz MD Faculty of Medicine, Division of Dermatology, University of British Columbia.

**Background:** The role of interferon- $\alpha$  (IFN- $\alpha$ ), ultraviolet B light (UVB), keratinocyte cell death in the development of systemic lupus erythematosus (SLE) were explored in an *in vivo* NOD murine model. Topical imiquimod (Aldara®, 3M), a Toll-like receptor 7 agonist, was used to induce a pro-inflammatory environment through the synthesis of IFN- $\alpha$ , while UVB was used to cause keratinocyte cell death. 5-fluorouracil (5FU) was used to promote cell death and also for its photo-sensitizing effects. **Methods:** Thirty mice were split into groups of six with the following treatment protocols: imiquimod alone, imiquimod / UVB / 5FU, UVB and imiquimod, UVB only, and a control group. Sera were then sampled bi-weekly and analyzed for anti-nuclear (ANA) and anti-dsDNA antibodies. **Results:** Treatment with either UVB alone, in combination (with 5FU & imiquimod) or imiquimod alone resulted in ANA seroconversion. At higher ANA titres, mice treated with 5FU / UVB / imiquimod and UVB / imiquimod showed the highest rates of ANA

seroconversion at titres of 1:160 and 1:320 ( $p < 0.05$ ). In addition, mice given both imiquimod and UVB therapy and imiquimod in combination with UVB and 5FU showed greater rates of anti-dsDNA seroconversion at rates of 100% and 83%, respectively ( $p < 0.01$ ). **Conclusion:** In conclusion, UVB light and IFN- $\alpha$ , with or without photosensitization may accelerate lupus-like autoimmunity.

**(11:22am)**

**DIFFERENTIAL CYTOKINE PRODUCTION IN PSORIASIS AND PSORIATIC ARTHRITIS.**

Marcie Ulmer MD,<sup>1</sup> Jack Toole MD,<sup>2</sup> Cheryl Barnabe MD,<sup>3</sup> Carol Hitchon MD,<sup>4</sup> and Hani El-Gabalawy MD<sup>5</sup>. <sup>1</sup>Division of Dermatology, University of British Columbia, Vancouver, Canada <sup>2</sup>Division of Dermatology, University of Manitoba, Winnipeg, Canada. <sup>3</sup>Department of Internal Medicine, University of Manitoba, Winnipeg, Canada. <sup>4,5</sup>Division of Rheumatology, University of Manitoba, Winnipeg, Canada.

**Introduction:** Psoriasis is a chronic immune-mediated skin disorder characterized by inflammation and abnormal epidermal proliferation. It is now known that T cells and cytokines play central roles in the initiation and maintenance of this disease. They influence the migration of inflammatory cells into the skin and increase the activity of keratinocytes. This disease is common and affects approximately 2-3% of the general population. At least seven to ten percent of those affected with psoriasis will also be afflicted by psoriatic arthritis. Psoriatic arthritis is an inflammatory arthritis with three subsets. The disease is thought to be significantly under diagnosed. Morbidity occurs in both psoriasis and psoriatic arthritis from extensive skin involvement and disability from joint disease. Early therapeutic intervention can reduce the morbidity related to these conditions and thus prompt diagnosis of psoriatic arthritis is essential. The association between psoriasis and psoriatic arthritis is not clearly understood. This has led to interest in studying the immunologic characteristics of psoriasis and psoriatic arthritis. Both are T-cell mediated diseases with predominance of Th-1 helper cells. Despite similar cytokine profiles, differences do exist. **Purpose and objectives:** To identify different cytokine expression profiles in patients with psoriasis and psoriatic arthritis. This knowledge could lead to improved prediction of psoriasis patients who will develop psoriatic arthritis. This would lead to earlier diagnosis, resulting in earlier management of joint disease with less morbidity in a chronic disease.

**(9:18am)**

**ROLE OF PUMA AND P-AKT IN MELANOMA CELL GROWTH, APOPTOSIS, AND PATIENT SURVIVAL.** Alison M. Karst, Derek L. Dai, Vincent Ho, and Gang Li. Department of Medicine, Division of Dermatology, Vancouver Coastal Health Research Institute, University of British Columbia.

Malignant melanoma is an aggressive and chemoresistant form of skin cancer characterized by rapid metastasis and subsequently, poor patient prognosis. The development of innovative

therapies with improved efficacy is critical for the treatment of this disease. We show, using tissue microarray analysis, that patients with both weak PUMA and strong p-Akt expression in melanoma tissue had significantly worse 5-year survival than patients having either weak PUMA or strong p-Akt expression alone ( $P < 0.001$ ). Importantly, no patients having both strong PUMA and weak p-Akt expression in primary tumor tissue died within 5 years. We propose a two-pronged therapeutic strategy of (1) inducing PUMA expression and (2) inhibiting p-Akt phosphorylation. We have constructed a recombinant adenovirus containing human PUMA $\alpha$  cDNA and here, we report on its ability to inhibit melanoma cell growth. We show that ad-PUMA induces apoptosis and inhibits long-term survival of human melanoma cells *in vitro* via rapid activation of mitochondrial-mediated apoptosis. We also demonstrate that ad-PUMA treatment of human melanoma tumors significantly slows their rate of growth *in vivo*, using a SCID mouse model. In human melanoma cells with strong p-Akt expression, we show that inhibition of p-Akt using the synthetic phosphatidylinositol analogue, SH-5, decreases melanoma cell survival in a dose and time-dependent manner. Finally, we demonstrate that inhibition of p-Akt enhances ad-PUMA-induced apoptosis. Our results suggest that a strategy to correct the dysregulation of both PUMA and p-Akt expression in malignant melanoma may be an effective therapeutic approach to treatment of this disease.

(9:30am)

**EFFECTIVENESS OF PSORIASIS TREATMENT IN A DAY CARE PROGRAM: A PASI ANALYSIS.** Junling Zhang, Elaine Stebbing, Harvey Lui, Jerry Shapiro, Youwen Zhou. Division of Dermatology, Department of Medicine, University of British Columbia.

**Background:** It is well established that day care treatment programs are effective for managing psoriasis. However, the effectiveness has not been well documented using standardized evaluation parameters. **Objectives:** The goal of this study is to analyze the efficacy of a psoriasis day care program using modified Psoriasis Area and Severity Index (PASI) as a benchmark. **Methods:** Total 132 psoriasis patients treated in the Vancouver Psoriasis Day Care Center in the past two years evaluated. They received combined UVB phototherapy with topical treatments such as anthralin, tar, and /or corticosteroids over a two-week period. A physician global assessment (PGA) scale (0% for no improvement to 100% for complete improvement) was used to evaluation treatment effect for all patients. In addition, the baseline and Day 10 psoriasis area and severity index (PASI) were assessed for 64 patients. **Results:** The average baseline PASI was 13.6. By Day 10, there was a 68.1% reduction in psoriasis severity based on PGA using a scale of 0% (no improvement) to 100% (remission). Based on PASI analysis, the average PASI reduction by Day 10 was 59.6%, with 75% patients achieving greater than 50% reduction of PASI, 30% patients achieving greater than 75% reduction of PASI, and 3% having greater than 90% PASI reduction. **Conclusion:** With an average day-10 reduction of PASI of 59.6%, a day care program combining UVB and topical treatments over a two-week period seems to be a rapid and effective therapy for treating moderate to severe psoriasis.

(9:42am)

**PRELIMINARY MICRO-RAMAN IMAGES OF NORMAL AND MALIGNANT HUMAN SKIN CELLS.** Michael A. Short<sup>a</sup>, Harvey Lui<sup>b</sup>, David I. McLean<sup>b</sup>, Haishan Zeng<sup>c</sup>, Michael X. Chen<sup>a</sup>.  
<sup>a</sup>Department of Physics, Simon Fraser University, 8888 University Drive, Burnaby, B.C., Canada V5A 1S6; <sup>b</sup>Division of Dermatology, University of British Columbia, 835 West 10th Avenue, Vancouver, B.C., Canada V5Z 4E8; <sup>c</sup>Cancer Imaging Department, British Columbia Cancer Research Centre, 601 West 10<sup>th</sup> Avenue, Vancouver, B.C., Canada.

Micro-Raman spectroscopy covering a frequency range from 200 to 4000  $\text{cm}^{-1}$  was used to image human skin melanocytes and keratinocytes with a spatial resolution of 0.5  $\mu\text{m}$ . The cells were either cultivated on glass microscope slides or were located within thin sections of skin biopsies mounted on low fluorescence BaF2. A commercially available system was used to obtain the spectra utilizing a x100 long working distance objective with a numerical aperture of 0.8, and a cooled CCD. Both 633 and 515 nm excitations were tried, although the latter proved to be more efficient at producing Raman emission mostly due to the  $1/r^4$  dependence in light scattering. Fluorescence emission from the cells was surprisingly low. However, the excitation power at the sample had to be kept below about 2 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. Spectra from purified DNA, RNA, lipids, proteins and melanin were obtained and these spectra were compared with the skin cell spectra with the aim of understanding how they are distributed over a cell and how the distribution changes between different cells.

(1:30pm)

**COLORIMETRIC MONITORING OF POSTINFLAMMATORY PIGMENTATION: PRELIMINARY OBSERVATIONS.** Al Robaee A, Al Khayat H, Zhao J, Zeng H, McLean DI, Lui H. Division of Dermatology, University of British Columbia and Department of Cancer Imaging, British Columbia Cancer Agency, Vancouver, BC.

**Background and Objectives:** Although melanin is believed to be the main source of skin discoloration in postinflammatory hyperpigmentation (PIH), recent spectroscopic analyses suggest that hemoglobin can also contribute significantly to the perception of skin darkening, especially during the early phases of PIH. The objectives of this study are to develop a system for the quantitative measurement of PIH and to identify the biophysical basis for PIH. **Patients and Methods:** The study was designed to assess the PIH by colorimetry using the CIE (Commission Internationale de l'Eclairage)  $L^*a^*b^*$  system. The PIH and adjacent normal skin were assessed noninvasively using a handheld chromameter (Minolta CM-2600D Spectrophotometer). A single unit difference in the  $L^*a^*b^*$  coordinate system represents the minimal perceptible color difference that can be perceived by the human eye. **Results:** Twenty-four patients have been enrolled to date, with an age range between 18 and 73 years. Fifteen were males and nine were females; all had type I, III or IV skin. The causes of PIH were

inflammatory dermatoses (13), trauma (6), laser therapy (3), and burn (2). The PIH were located in extremities (13), face (7), and trunk (4). The overall L\*a\*b\* color difference between PIH and adjacent normal skin was  $9.6 \pm 5.3$ . The corresponding differences in L\*, a\*, and b\* were  $-8.6 \pm 5.0$ ,  $2.0 \pm 2.2$ , and  $-1.5 \pm 3.5$  respectively. These differences decreased in absolute value over time on a scale of weeks to months. **Conclusion:** The color changes in PIH over time can be quantified using a handheld chromameter; detailed characterization of the biophysical basis for PIH will require further study with spectroscopic techniques.

(1:42pm)

**A NEW SPECTROMETER FOR MEASURING EXCITATION-EMISSION MATRICES OF SKIN AUTOFLUORESCENCE.** D. Thiessen, H. Lui, H. Zeng. LAMP - The Laboratory for Advanced Medical Photonics BC Cancer Agency, UBC Division of Dermatology, and Vancouver Coastal Health Research Institute.

**Background:** Tissue autofluorescence is an optical property whereby intact biological materials absorb light and then re-emit it at longer wavelengths. Fluorescence spectra are determined by the biochemical structure and composition of specific fluorescing compounds present within tissue known as fluorophores. The emission spectrum is also dependent on the wavelength of the excitation light. By systematically measuring successive emission spectra over a range of excitation wavelengths, a 3-D graph, known as an excitation-emission matrix (EEM), can be produced. **Objective:** Our goal is to construct a system for clinical cutaneous EEM spectroscopy measurements which can be applied to the diagnosis of skin diseases. **Design & Results:** We have developed a fast EEM spectrometer for measuring skin autofluorescence. The system uses acousto-optical tunable filters which can isolate any wavelength within a 550-950nm range and are capable of switching wavelengths on a time scale down to one millisecond. The data is collected non-invasively through a fibre-optic probe. Preliminary measurements show notable differences in spectra measured from different skin lesions. **Conclusion:** EEMs provide a composite picture of all the fluorophores within a given tissue and potentially yield far more diagnostic information than conventional single-wavelength-excited emission spectra. These devices are expected to complement other diagnostic techniques and tools used for non-invasive diagnosis of skin disease.

(1:54pm)

**REDUCED BIM EXPRESSION IN HUMAN MELANOMAS.** Derek L. Dai,<sup>1</sup> Magdalena Martinka,<sup>2</sup> Vincent Ho<sup>1</sup> and Gang Li<sup>1</sup>. <sup>1</sup>Department of Medicine, Division of Dermatology; <sup>2</sup>Department of Pathology, Vancouver Hospital and Health Sciences Centre, University of British Columbia, Vancouver, BC, Canada.

Malignant melanoma is a life-threatening skin cancer due to its highly metastatic character and resistance to radio- and chemotherapy. It is believed that the ability to evade apoptosis is the key mechanism for the rapid growth of cancer cells. However, the exact mechanism for failure in

the apoptotic pathway in melanoma cells is unclear. In this study, we sought to determine whether pro-apoptotic protein Bim contributes to human melanoma progression and survival. We used tissue microarray and immunohistochemistry to examine Bim expression in 52 cases of dysplastic nevi, 164 cases of primary melanomas, and 54 cases of metastatic melanomas. Here we report that reduced Bim expression was observed in 35%, 27%, and 63% of the biopsies in dysplastic nevi, primary melanoma, and metastatic melanomas, respectively. Significant differences for Bim staining pattern were observed between dysplastic nevi and metastatic melanomas ( $P < 0.01$ ,  $\chi^2$  test), and between primary melanomas and metastatic melanomas ( $P < 0.001$ ,  $\chi^2$  test). There was no significant difference for the staining pattern between dysplastic nevi and primary melanomas ( $P > 0.05$ ,  $\chi^2$  test). Furthermore, our Kaplan-Meier survival curves showed that reduced Bim expression was correlated with both overall and disease-specific 5-year survival of melanoma patients ( $P < 0.001$  and  $P < 0.005$ , respectively, log-rank test). Taken together, our data indicated that Bim expression was reduced in human melanoma and that Bim may serve as a therapeutic target in melanoma.

**(2:06pm)**

**FAR-RED AND NEAR-INFRARED FLUORESCENCE IMAGING SYSTEM FOR NON-INVASIVE SKIN DIAGNOSIS AND EVALUATION.** Xiao Han, Harvey Lui, David McLean, Haishan Zeng. LAMP – The Laboratory for Advanced Medical Photonics. Cancer Imaging Department, BC Cancer Research Centre; UBC Division of Dermatology; and Vancouver Coastal Health Research Institute.

**Background and Objective:** Far-red and near-infrared (NIR) fluorescence has recently been observed from a couple of human tissue types and demonstrated potential uses for optical diagnosis. However, there is not enough study of applying this technique for skin in vivo. The objective of this study is to develop a macroscopic, far-red and NIR fluorescence imaging system for non-invasive skin diagnosis and evaluation. **Materials and Methods:** Light coming from a xenon arc lamp is coupled into a ring fiber light guide to achieve uniform illumination on tissue surface. A CCD camera with high sensitivity in far-red and NIR is used for image acquisition. Various excitation (628 nm, 684 nm, 785 nm, etc.) and corresponding emission filters can be inserted to obtain images in desired spectral regions. The system can also perform polarized imaging in both fluorescence and reflectance mode. **Results and Conclusion:** Fluorescence images of skin lesions up to ~1 inch diameter can be acquired in the 600-1000 nm region and good S/N images can be conveniently obtained with exposure time of less than 1 second. The system is now being used in an outpatient dermatology clinic for data acquisition of various skin diseases including skin cancer.

(2:18pm)

**TOPICAL CPG PROMOTES CROSS PRESENTATION OF ANTIGEN AND GENERATES AN EFFICIENT MEMORY RESPONSE.** Hossain M Najar and Jan P Dutz. Department of Medicine and CF Research Institute of Children and Women's Health, University British Columbia, Vancouver, British Columbia, Canada.

Skin hosts dendritic cells (DC) which upon activation, are able to cross-present soluble antigen in the context of MHC I to CD8<sup>+</sup> T cells and generate potent cytotoxic T cells (CTL). We have shown that activation of these DC in the skin by applying oligonucleotide adjuvant CpG epicutaneously (epi- that means on the skin) will generate a robust CTL response to injected soluble foreign antigen. We also found that epi application of CpG generates an enhanced CTL memory and effector response when compared to s.c route of CpG introduction. Mice immunized with ovalbumin (OVA) and either epi or subcutaneous CpG as adjuvant were compared with regards to OVA –specific CTL responses. We compared the effector and memory phenotype of OVA-specific CTL by determining CD127 (IL-7 $\alpha$  receptor) and CD 43 molecule expression at different time-points. Comparison of effector phenotype (CD127<sup>lo</sup>, CD43<sup>hi</sup>) on day 14 and a memory phenotype (CD127<sup>hi</sup>, CD43<sup>lo</sup>) on day 45 among OVA-specific CD8<sup>+</sup> T cells in spleen between different methods of adjuvant application reveals that epic application of CpG induces more memory CTL and a higher effector CTL population at early time-points than sc administration of adjuvant. Thus topical administration of CpG adjuvant is superior to subcutaneous administration of CpG adjuvant when used in combination with standard vaccine protocols. These results further suggest that the induction of cross-presentation of viral and modified self antigens (and consequently CTL induction to these antigens) may be accomplished by the use of DCs in the skin by topical application of CpG.

(2:30pm)

**MULTIMODE SPECTROSCOPY FOR THE ASSESSMENT OF POST-INFLAMMATORY PIGMENTATION *IN VIVO*.** Jianhua Zhao<sup>a,b</sup>, Hana Alkhayat<sup>b</sup>, Ahmad Al Robaee<sup>b</sup>, Haishan Zeng<sup>a,b</sup> David I. McLean<sup>a,b</sup>, and Harvey Lui<sup>a,b</sup>. <sup>a</sup> LAMP – The Laboratory for Advanced Medical Photonics, Cancer Imaging Department, BC Cancer Research Centre, 675 West 10th Ave, Vancouver, BC Canada V5Z 1L3; <sup>b</sup> LAMP – The Laboratory for Advanced Medical Photonics, UBC Division of Dermatology and Vancouver Coastal Health Research Institute, 835 West 10th Ave., Vancouver, BC Canada V5Z 4E8.

**Background:** Post-inflammatory pigmentation (PIH) is a common acquired skin disease, which is particularly disfiguring when it occurs on exposed skin sites such as the face or hand. The mechanism of post-inflammatory pigmentation still remains unclear. Clinical assessment and investigation of PIH is largely based on visual examination. Various optical spectroscopic techniques including diffuse reflectance spectroscopy, fluorescence spectroscopy and Raman spectroscopy have the capability of probing the morphologic and biochemical properties of normal and diseased skin. In this study, we assess the biochemical and morphologic changes associated with PIH using the above optical spectroscopic techniques. **Methods:** Twelve patients of skin type III-VI with PIH have been evaluated to date. The diffuse reflectance

spectrum, the fluorescence spectrum and the Raman spectrum were obtained using custom-built reflectance/fluorescence fiber spectrometer and the rapid Raman systems. For paired analysis, both pigmented skin and the adjacent normal skin were measured. A Konica Minolta Chromameter (Model CM-2600d) was used to record the color of the PIH and normal skin. The diffuse reflectance spectrum was also converted into the CIE standard L\*a\*b\* color system. The melanin and the hemoglobin content in the PIH were quantitatively derived from the diffusive reflectance spectrum using the Stamatas-Kollias algorithm. **Results and Conclusions:** The Raman, fluorescence and reflectance spectra were analyzed and distinctive spectral features were observed indicating that the biochemical and morphologic properties were changed associated with PIH. We found that PIH always has a lower value in L\*, more melanin and oxy-hemoglobin content which is consistent with clinical appearance as perceived by the eye.

(2:42pm)

**SHORT CONTACT TOPICAL 5-AMINOLEVULINIC ACID PHOTODYNAMIC THERAPY (SCALP) FOR ACTINIC KERATOSES (AKs): NOVEL PROTOCOL AND STUDY TO DETERMINE EFFICACY, CONVENIENCE AND COMFORT.** Julian A. Hancock FRCP(C), Elizabeth Harrison RN, Dione Brown BSN, Dermatologist Skin Laser Clinic, Nanaimo, BC.

**Background:** SCALP reduces AKs, improves skin appearance, and should reduce age related demographic increases in non-melanoma skin cancer (NMSC). Objectives: Determine patient experiences concerning efficacy, comfort and convenience of novel SCALP protocol, record comparisons to Liquid Nitrogen (LN2), topical 5-Fluorouracil (5FU) and topical Imiquimod (ALD) therapies, and note cosmetic skin improvement. **Method:** Retrospective questionnaire survey of 39/137 SCALP patients treated in a private Vancouver Island Dermatology Clinic over a 14 month period; 10 questions were put to each subject and responses recorded. Novel SCALP protocol features two hour incubation of face/scalp with Levulan (Dusa Labs) then exposure to 412-422nm blue light (BLU-U) under supervision, followed by ELOS (Syneron) pulsed light with radiofrequency to face only. **Results:** 36/39 (92%) experienced > 50% reduction in face/scalp AKs. 28/39 (77%) experienced > 70% reduction in face/scalp AKs. 31/39 (79%) felt SCALP more effective than LN2. 33/39 (84%) experienced cosmetic improvement in facial skin. 38/40 (97%) experienced no adverse effects to skin. **Limitations:** Size of retrospective study, reliance on subjective impressions and recall of procedure. **Conclusions:** The Nanaimo SCALP protocol demonstrates 'field' reduction of AKs in an effective, comfortable and convenient manner with cosmetic skin improvement, promising to reduce NMSC rates thereby improving patient morbidity and mortality as well as Healthcare issues.

(2:54pm)

**ING3 PROMOTES UV-INDUCED APOPTOSIS VIA FAS/CASPASE-8 PATHWAY IN MELANOMA CELLS.** Yemin Wang and Gang Li. Division of Dermatology, University of British Columbia, Vancouver, BC V6H 3Z6.

The novel ING tumor suppressor family proteins (ING1-5) have been recognized as the regulators of transcription, cell cycle checkpoints, DNA repair, apoptosis, cellular senescence, angiogenesis and nuclear phosphoinositide signaling during the past decade. ING proteins contain a few conserved domains, including plant homeodomain (PHD) motif, nuclear localization signal (NLS), and potential chromatin regulatory (PCR) domain, suggesting that they may share common biological functions. ING3 has been shown to mediate p53-dependent transcription, cell cycle and apoptosis, possibly by modulating the NuA4 complex histone acetyltransferase activity. Since ING1b and ING2 have been shown to be involved in cellular stress responses such as nucleotide excision repair and apoptosis after UV irradiation, we investigated whether ING3 also mediates UV-induced apoptosis. We found that the ING3 was rapidly induced by UV irradiation. Using the stable clones of melanoma cells overexpressing ING3, we showed that overexpression of ING3 significantly promoted UV-induced apoptosis. Unlike its homologues ING1b and ING2, ING3-mediated apoptosis was independent of functional p53. Furthermore, ING3 did not have effect on the expression of mitochondrial proteins, but induced the cleavage of bid, caspases-8, -9, and -3. Moreover, ING3-mediated apoptosis was inhibited by blocking the recruitment of Fas-associated Death Domain (FADD) or caspase-8. In addition, we detected more fas expression in ING3 stable clone F5 cells and knockdown of fas can abolish ING3-mediated apoptosis, indicating that ING3 enhances UV-induced apoptosis via activating the fas/caspase-8 pathway

**(3:06pm)**

**RESEARCH PROPOSAL: SURVEY OF RECENT CANADIAN DERMATOLOGY GRADUATES.** Stephanie J. Côté, Najwa Somani, and Richard I. Crawford, University of British Columbia.

**Background:** Despite workforce shortages, there are relatively few dermatologists trained every year in Canada. A Canadian dermatology workforce survey conducted in 2001 confirmed this reality and provided information about the demographics, workload, and future career plans of dermatologists in this country. An appraisal of dermatology residency training in 2004 provided data to assist dermatology programs with the improvement of their curricula. No prior studies have evaluated how recent dermatology graduates perceive their residency to have prepared them for their careers, nor has any previous study examined the demographics and practice components of this group. **Hypotheses:** 1) Current residency programs provide a broad-based training curriculum; and 2) Components of recent graduates' current practices differ from what they had perceived during residency that these would subsequently be.

**Objectives:** 1) To identify the basic demographics and practice components of recently graduated Canadian dermatology residents; 2) To characterize additional formal and informal training completed after residency; 3) To assess their perceived adequacy of residency training in preparing them for their current endeavors; 4) To provide suggestions for improvement in residency training programs; and 5) To help evaluate whether areas of need are being filled in Canada. **Methods:** Anonymous survey of Canadian dermatology graduates from the classes of 2001-2005.