Oral Presentations

(8:30am)

SCF^{Skp2}-MEDIATED ING3 DEGRADATION REGULATES CELL CYCLE CONTROL IN MELANOMA CELLS

Guangdi Chen,[1] Yemin Wang,[1] Marco Garate,[1] Jianwei Zhou,[2] Gang Li[1]
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The novel tumor suppressor ING3 has been shown to modulate transcription, cell cycle control, and apoptosis. Nuclear ING3 expression was remarkably reduced in human melanomas, and reduced nuclear ING3 was significantly correlated with increased cytoplasmic ING3 level and a poorer patient survival. Here we show that overall ING3 expression is decreased in metastatic melanoma cells due to a rapid protein turnover. Further studies demonstrate that ING3 undergoes proteolysis via the ubiquitin-proteasome pathway. ING3 physically interacts with SCF^{Skp2} E3 ligase complex. Knockdown of Cul1 or Skp2 reduces the ubiquitination of ING3 and significantly stabilizes ING3 in melanoma cells. Lysine 96 residue of ING3 is essential for its ubiquitination as its mutation to arginine dramatically abrogated ING3 ubiquitination and protein turnover. Silencing Skp2 or overexpressing ING3 significantly arrested melanoma cells at G1-phase, while knockdown of ING3 decreased the distribution of melanoma cells at G1-phase. In addition, disruption of ING3 inhibited Skp2 knockdown-induced G1-phase arrest, whereas interruption of Skp2-mediated ING3 degradation by K96R mutation stimulated ING3-induced G1-phase arrest in melanoma cells. Taken together, our data reveal a key role of ubiquitin-proteasome degradation system in the regulation of ING3 turnover and its tumor suppressive function and a potential relevance of cancer therapy by interfering ING3 degradation.

(8:42am)

NQO1 INDUCES CELL CYCLE PROGRESSION IN MELANOMA CELLS

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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 in response to stresses including oxidants, UV and ionizing radiation. Its induction provides 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 melanoma cells can generate superoxide ion at the cellular membrane where it activates the nuclear factor kappa B and in turn induces cell proliferation. We hypothesize that the expression of NQO1 is associated with the cell cycle progression of melanoma cells. Working with a number of cell lines, we observed that NQO1 is overexpressed in most melanoma cell lines when compared to melanocytes. Cells expressing higher levels of NQO1 proliferate faster than NQO1-deficient cells. The ectopic expression of NQO1 in NQO1-deficient melanoma cells induces both the proliferation and the ability of these cells to form colonies in soft agar. The faster proliferation of these cells correlates with more rapidly progression from G1 checkpoint to S phase and upregulation of cyclin D. These results suggest that NQO1 could play a role in inducing melanoma pathogenesis. Clinical Significance and knowledge translation: This study will contribute to unveil the molecular mechanism of how NQO1 induces cell cycle progression and therefore may lead to novel strategies in the design of better therapies to treat melanoma patients.

(8:54am)

EXPRESSION OF THE QUINONE OXIDOREDUTASE 1 IN MELANOMA

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Purpose: Recent studies have suggested that deletion of the quinone oxidoreductase 1 (NQO1) can promote skin carcinogenesis. However, the effects of NQO1 in melanoma are still unknown. To investigate whether NQO1 plays a role in melanoma initiation and progression, we measured NQO1 expression in dysplastic nevi and primary melanoma biopsies and evaluated the prognosis value of NQO1 expression in human melanoma. Experimental Design: We used tissue microarray and immunochemistry to determine NQO1 expression in 56 dysplastic nevi and 93 primary melanomas, and analyzed the correlation between NQO1 expression and melanoma progression or 5-year patient survival. Results: Our data show that strong expression of NQO1 was detected in 32% of primary melanomas compared with 17% of dysplastic nevi ($p < 0.01$). Furthermore, NQO1 expression is lower in superficial spreading and nodular melanoma than other subtypes ($p < 0.001$). However, we found no significant correlation of NQO1 expression with other clinical parameters such as age, gender, tumor thickness, tumor ulceration and location of melanoma. Similarly, our data revealed that strong expression of NQO1 was not significantly correlated with 5-year patient survival ($p > 0.05$). Conclusion: NQO1 expression is significantly increased in primary melanomas compared with dysplastic nevi, implying that NQO1 may play an important role in melanoma initiation.

Clinical Significance and KT: This study leads to a better understanding of the role of NQO1 in melanoma pathogenesis and this molecule may be used as a marker for melanoma initiation.

(9:06am)

THE EXPRESSION OF A CANCER INFLAMMATION MARKER CORRELATES WITH MELANOMA INVASION AND PREDICTS POOR SURVIVAL IN STAGE III AND STAGE IV MELANOMA

Jiang H [1], Tan L [1, 2]*, Ip W [1,2], Su, M [1,2], Dai, D [1], Brasher, P [3], Zhang, Y [1,2], Wang, Y [1,2], McLean, D [1,2,4], Martinka, M [5], Li G 1 [1,2,4], and Zhou Y [1,2,4].
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Melanoma is one of the most aggressive and life-threatening cancers due to its resistance to conventional treatments and high metastatic potential. In tumor biopsies from various stages of melanoma examined through immunohistochemistry, we observed that an acute phase reactant protein was virtually absent in normal nevi, dysplastic nevi and melanoma *in situ* but its expression was dramatically increased as lesions progressed to primary and metastatic melanoma ($P<0.001$). The possibility this protein contributes to melanoma invasion and metastasis was further examined *in vitro* using cultured MMRU and KZ-28 melanoma cells. Down-regulation of this protein through siRNA knockdown leads to decreased invasion into Matrigel, but does not inhibit cell proliferation, migration, or survival. Kaplan-Meier curves were generated for the overall and disease specific survival of 159 patients with primary melanoma and 51 patients with metastatic disease. Using a validated 3-point scoring system, stained lesions from these patients were classified as exhibiting weak protein expression (score of 0 or 1) or strong expression (score of 2 or 3). Although expression levels of this protein did not correlate with worse 5-year survival in primary melanoma patients ($P>0.5$), increased expression strongly correlated with poorer survival in metastatic melanoma, with a mean survival of 46.2 months for patients with weakly stained lesions compared to 14.9 months for strongly stained lesions. Taken together, this acute phase reactant protein appears to contribute to melanoma invasion and metastasis and is a novel prognostic marker for metastatic melanoma.

(9:18am)

ROLE OF SWI/SNF CHROMATIN REMODELING COMPLEX IN NUCLEOTIDE EXCISION REPAIR IN HUMAN MELANOMA CELLS

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Melanoma is a life-threatening skin cancer, for its ability to metastasize rapidly and its resistance to radio/chemotherapy. Ultraviolet (UV) radiation is the major environmental factor for development of melanoma. It induces DNA lesions such as cyclobutane pyrimidine dimers (CPDs). CPD is repaired by nucleotide excision repair (NER) pathway, which involves up to thirty polypeptides. For this number of repair factors to gain access to the lesion sites, chromatin's compact structure needs to be relaxed. It is proposed that the relaxation process, or chromatin remodeling, precedes NER in order to provide favorable environment for recruitment of repair factors. Chromatin remodeling is allowed through SWI/SNF ATP-dependent chromatin remodeling complex. We recently found that SNF5, a core subunit of SWI/SNF complex, mRNA level is down-regulated in five human melanoma cell lines by at least 60% compared to melanocytes. We also found that overexpression of SNF5 is able to increase DNA repair efficiency by three folds in host-cell reactivation assay. To further study the role of SNF5 in NER, we established stable HEK293-cell line with SNF5 knockdown, and observed 20% lower repair rates in SNF5 knockdown cells compared with vector control in slot-western analysis of CPD. Finally, we found that UV-induced chromatin relaxation was abrogated in cells with SNF5 knockdown using Micrococcal nuclease digestion assay. Our data show the importance of SWI/SNF complex as chromatin remodeling factors in NER in melanomas. Understanding the molecular mechanisms of how UV-damaged DNA is repaired may eventually enable us to design effective strategies for skin cancer prevention.

(9:30am)

SCREENING FOR MELANOMA AND NON-MELANOMA SKIN CANCER WITH ANNUAL CUTANEOUS EXAMINATION: REASONS FOR NON-COMPLIANCE

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Background: Annual Cutaneous Examination (ACE) is recommended by many dermatologists as a screening method for early detection of melanoma and non-melanoma skin cancers in patients with increased risk for these lesions. Objective: To

explore patient compliance with a dermatologist's recommendation for ACE and to characterize reasons for non-compliance with this recommendation. Methods: A retrospective chart review was performed for patients seen by the same dermatologist over a 10-year period with the diagnosis of either benign neoplasm of the skin or melanoma. Charts were reviewed to determine if the recommendation was made for ACE. Patients found to be non-compliant with this recommendation were invited to complete a survey exploring reasons for non-compliance. Results: Nine hundred and fourteen charts were reviewed, 356 patients were targeted for ACE, and 215 of these patients were found to be non-compliant. Questionnaires were mailed to the non-compliant patients with a response rate of 32%. Over half of respondents reported at least one cutaneous examination since they saw the study dermatologist, and 26 % reported undergoing ACE. The most common reasons cited for non-compliance were; 1) self-monitoring of skin, 2) perceived lack of need for ACE, 3) being unaware of the recommendation for ACE, and 4) uncertainty about which physician would be responsible for follow-up. Thirteen percent of respondents were diagnosed with skin cancer since they last saw the study dermatologist. Conclusions: A failure to comprehend the recommendation and importance of ACE is a significant reason for non-compliance. Strategies that emphasize the benefit of ACE may improve this situation.

(9:42am)

SKIN CANCER DETECTION USING NONINVASIVE *IN VIVO* RAMAN SPECTROSCOPY – PRELIMINARY RESULTS

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As a non-invasive optical technique Raman spectroscopy can assess molecular structures and conformations within biological tissue. We developed a rapid real-time Raman spectrometer system with measurement times of less than 1 second suitable for clinical measurement. Patients with benign and/or malignant skin lesions were recruited. Both the lesional skin and its surrounding normal skin were measured using real-time Raman spectrometer. 256 cases were included in this study, of which there were 24 cases of basal cell carcinoma (BCC), 49 cases of squamous cell carcinoma (SCC), 37 cases of malignant melanoma (MM), 24 cases of actinic keratosis (AK), 53 cases of seborrbeic keratosis (SK), 32 cases of atypical nevus (AN), 22 cases of compound nevus (CN), 25 cases of intradermal nevus (IN), and 23 cases of junctional nevus (JN). The patients were divided into two categories for analysis: (1) skin cancer (BCC, SCC, MM, AK) versus benign lesions (SK, AN, CN, IN, JN); (2) MM versus benign pigmented lesions (SK, AN, CN, IN, JN). The Raman spectra were analyzed using multivariate partial least squares

and linear discriminant analysis. Statistical analysis demonstrated that skin cancers could be well discriminated from benign skin lesions (AUC of the ROC curve = 0.906, sensitivity = 91%, specificity = 75%); and malignant melanoma from benign pigmented lesions (AUC of the ROC curve = 0.930, sensitivity = 97%, specificity = 78%). The results demonstrated that real-time bedside Raman spectroscopy is both technically feasible and capable of assisting with *non-invasive* skin cancer diagnosis.

(11:00am)

SKIN LESION SEGMENTATION USING THE GRAY-CONVERSION BY A CUSTOMIZED COLORMAP

Maryam Sadeghi[1], Tim K. Lee[1,2] and M. Stella Atkins[1]
1: School of Computing Science, Simon Fraser University. 2: Department of Dermatology and Skin Science, University of British Columbia

Lesion segmentation is a key step in the automatic classification of moles and skin cancer diagnosis. We developed a novel automatic segmentation algorithms based on converting an indexed image to the gray level using a customized colormap. Our method is a low-cost accurate approach for the difficult cases that can get desirable results for images which are not easy to segment such as partial images of lesions, dark-skin lesions, lesions with smoothed borders, etc. We demonstrate that converting images from index type to grey-scale using different colormaps will make the intensity differences clearer and improves the accuracy of segmentation process. In addition, to get a good segmentation result independent of skin colors, we worked on an automatic threshold detection method to use for customized colormaps. We found the blue channel of RGB as the optimum color-space and color channel to use in our work. We can take the spatial arrangement of lesion pixels into account during the segmentation to enhance the method performance. Our proposed method allows a fast processing and it will be robust to geometric variations of skin lesion patterns, partial occlusion and resolution changes. Clinical Significance and KT: Our method is a fully automated segmentation procedures for skin images that successfully segment a large number of skin lesion and it can be used as the most important stage in the analysis of moleskin lesions and automated skin cancer diagnostic systems.

(11:12am)

SKIN MOLE MATCHING INCORPORATING TEMPLATE-NORMALIZED COORDINATES

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Density of moles is a strong predictor of malignant melanoma. Some dermatologists advocate periodic full-body scan for high-risk patients. In the current practice, the physician compares the images at different time instances to recognize changes. There is an important clinical need to follow changes in the number of moles and their appearance in images from two different times. To prepare an automatic tracking system, we propose the first human back template allowing an anatomically meaningful comparison between moles in the human back images. The template is constructed based on a predefined number of longitudes and latitudes passed through anatomically meaningful landmarks. After extracting normalized mole location coordinates, we propose using an automatic graph-based approach for finding corresponding moles. We evaluate our proposed method on 56 pairs of real dermatological images. The results show that: first, using our proposed anatomy based normalized coordinates for the matching algorithms, we substantially improve the mole matching accuracy. Second, our proposed matching algorithm compares favourably with the state-of-the-art. Clinical Significance and KT: Visual inspection of moles is costly, time consuming, and may be error prone due to user fatigue. Therefore, preparing such an automatic mole tracking system has benefits especially for patients who are at a high risk of developing melanoma and, hence, require regular mole examinations.

(11:24am)

IN-VIVO SKIN ROUGHNESS MEASUREMENTS OF DORSAL HAND AND VOLAR FOREARM USING LASER SPECKLE

Bernardita Policarpio[1], Lioudmila Tchvialeva[1], Harvey Lui[1,2], David I. McLean[1], Haishan Zeng[1.2], Tim K. Lee[1,2]

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Introduction: Skin topography is of great interest for many dermatologists and skin scientists. We are developing a novel method for *in-vivo* skin roughness measurement in about 5 msec by analysing laser speckles. The purpose of this study is to evaluate the effectiveness of the device. Methods: To validate the device which derives the root-mean-square roughness from the speckle contrast, we used it to measure a group of volunteers. The same skin location was measured thrice and the average skin roughness was then computed. Results: We recruited 40 volunteers. The first group was 31 patients who attended a skin clinic in the Skin Care Centre, Vancouver General Hospital. We acquired the normal skin roughness of the corresponding sites that required medical attention. The second group was nine volunteers. The same spot of their right dorsal hand and right volar forearm was measured. When we compared all our measurements on various body sites with the literature values, a reasonable correlation was found (Pearson correlation coefficient = 0.64 and Spearman correlation coefficient = 0.67). For the subgroup of 9, the mean roughness measurement of the dorsal hand was 20 ± 4 um

while that of the volar forearm was 16 ± 5 um. Results showed a significant increase in skin roughness on the dorsal hand as compared to the volar forearm (p=0.038). Conclusions: We have demonstrated that our roughness measurements were in agreement with published values; furthermore this instrument can detect a significant difference in the roughness measurements between the dorsal hand and volar forearm.

(11:36am)

A VISUAL AID FOR DEFINING AND IDENTIFYING DERMOSCOPIC STRUCTURES

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We seek to explicitly define dermoscopic structures in skin lesions. This would not only improve automated diagnostic techniques, but may also lead to clinically relevant applications. We have developed a framework sufficiently general to define all dermoscopic structures. We demonstrate the framework's ability by identifying the structure 'pigment network' and differentiating between typical and atypical variants. Our dataset consists of 94 images from an atlas of dermoscopy that are labeled as having either an 'absent', 'regular' or 'irregular' pigment network. Locational information is not specified, only that it occurs somewhere in the image. We begin by performing various pixel-wise measurements on these images such as filter-bank convolutions, wavelet decompositions, etc. Multivariate distributions over these measurements are then constructed and maximum likelihood estimation is used to label unseen images. The system can detect pigment networks with 87% accuracy. It also identifies the type of pigment network with 72% accuracy. The framework also creates visualizations showing the location of a particular dermoscopic structure, along with the associated certainty.
In conclusion, explicitly defining dermoscopic structures in statistical terms is a promising technique for both identification and visualization. Our study falls into the KT category: Early experiments with well defined objectives/hypotheses. It is hoped that the visualizations will improve identification and training methods. Additionally, we envision a system whereby experts can interact with the visualization and re-label portions they disagree with. Such locational information would improve the overall accuracy of the system, and serve as a starting point to build consensus amongst experts.

(11:48am)

PERSISTENT FACIAL PSORIASIFORM DERMATITIS: A NEWLY DESCRIBED DERMATOSIS DISCOVERED THROUGH MODERN LIVE PATIENT TEACHING ROUNDS

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Introduction: A novel pattern of comparable cases has arisen from our departmental live patient presentation rounds at the University of British Columbia. These cases had the following constellation of features: i) facial preference; ii) persistent, discrete, scattered, salmon-coloured, thin, and round to oval papules with fine white scale; iii) histology resembling psoriasis or seborrheic dermatitis; iv) resistance to a range of anti-inflammatory therapies. Methods: Between 1996 to 2008, eleven patients were identified from our database amongst those who had been referred to our weekly university case rounds with an undiagnosed refractory facial dermatosis. Of these eleven patients, two were excluded because histopathology was not compatible, and one patient declined to have their clinical data included in this series. Results: The mean age of the eight patients was 38.4 years (range: 21-70 years). All cases revealed scattered well demarcated salmon-coloured, round to oval, 0.5-1.0 centimeter thin papules with fine white scale. Seven biopsies taken from six patients all showed psoriasiform epidermal hyperplasia with prominent parakeratosis, follicular plugging, and a purely lymphocytic superficial perivascular infiltrate without evidence of an interface dermatitis. The lesions have been resistant to topical therapy, phototherapy and systemic medications. Conclusion: This clinical pattern has been named persistent facial psoriasiform dermatitis and has not been previously reported in the literature to our knowledge. The maintenance of teaching rounds along with their formal documentation can provide a means for detecting and characterizing previously unidentified and perhaps less common pathological conditions periodically encountered by experienced clinicians.

(12:00noon)

CUTANEOUS DISEASE BURDEN AND DEMOGRAPHIC CHARACTERISTICS OF AN URBAN CANADIAN HIV DERMATOLOGY PRACTICE

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Background: The introduction of highly active antiretroviral agents (HAART), and subsequent advancement of newer agents and combination therapy has changed the frequency of cutaneous disease in individuals carrying HIV. Objective: The purpose of our study was to assess demographic characteristics, severity of immunosuppression,

and frequency of dermatologic disorders presenting to a Canadian specialized HIV dermatology practice. Methods: A cross sectional study was performed of 183 consecutive outpatient and inpatient consultations to a single HIV dermatology practice between January 2007 to December 2008. Results: The average age of patients was 45 years. 163 (88%) of the patients were male. The average CD4 count was 338 cells per microlitre (cpm). CD4 counts were 100 cpm or less in 18 patients. 46 of the patients seen were not on antiretroviral therapy. Multiple skin diseases were diagnosed in 120 patients (66%). Verrucae was the most common diagnosis, seen in 29 patients. The second most common diagnosis was dermatophyte infection, seen in 27 patients. There were 8 cases of Kaposi's sarcoma, 5 of eosinophilic folliculitis, and a single instance of oral hairy leukoplakia. Conclusions: Cutaneous infections were the most common diagnosis in this cross section. Classically described HIV dermatoses occurred in a low frequency in this patient group, which may reflect more successful management in the modern antiretroviral era.

(12:12pm)

USE OF ISLAND PEDICLE FLAP FOR RECONSTRUCTION OF LARGE DEFECTS OF THE MEDIAL CHEEK

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Background: Skin cancer represent the most common malignancy in humans. The most likely location for skin cancer is the face. After surgical excision of the tumor, special care must be taken to provide optimal cosmetic as well as functional reconstruction. Large defects of the medial cheek present a unique challenge to the reconstructive surgeon due to their propensity to develop ectropion of ipsilateral lower eyelid following reconstruction. Traditional reconstructive options include cheek rotation flap and full thickness skin graft. Objective: To present our experience with the use of island pedicle flap in reconstruction of large post-resection defects on the medial cheek. Methods: The study was carried out in University of British Columbia Skin Cancer Center. Twenty patients with large (>10cm^2) defects, seen in 2006-2008, were included in the study. The defects resulting from tumor resection using Mohs Micrographic Surgery (MMS) were repaired using the island pedicle flap technique. Final cosmetic and functional results were analyzed after a follow-up of 6 months to 2 years. Results: Good to excellent cosmetic results with functional preservation of lower eyelid patency was achieved in our patients. Conclusion: Large defects of the medial cheek are amenable to reconstruction with the island pedicle flap technique.

(1:30pm)

DEVELOPMENT OF NOVEL TOOLS TO STUDY EX VIVO T CELL SUBSETS IN HUMAN SKIN

Crome, Sarah[2], Broady, Raewyn[1], Kang, Christine[2], Yu, Jessie[1], Dutz, Jan[3] and Levings, Megan[2].
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Pathogenic immune responses in the skin are often attributed to altered numbers and/or function of distinct subsets of $CD4^+$ T cells and can result in inflammatory diseases such as psoriasis, scleroderma and atopic dermatitis. For example, either the absence of skin-homing T regulatory (Treg) cells, or a tissue-specific increase in inflammatory IL-17-producing T helper 17 (Th17) cells, can result in cutaneous inflammation. Thus development of methods to study $CD4^+$ T cell subsets in tissues such as the skin is critical to better define the mechanistic basis for chronic inflammatory diseases. We have established a method to isolate *in vivo*-differentiated Th17 cells from human peripheral blood by sorting on Th17 cell markers. We have fully characterized the resulting cells for common Th17-associated phenotypes and functions and are able to expand the Th17 cells, and Th1 control cells, in long-term cultures. In order to delineate the function of *ex vivo* Th1 and Th17 cells in human skin, we developed a modified skin explant model that involves culture of 4 mm punch biopsies of human skin with *ex vivo* Th17 cells. Preliminary data suggest that Th17 cells cause significant tissue destruction in this model, and experiments are ongoing to determine whether Treg cells can counteract these effects. These studies will define the role of T cell subsets in human skin inflammation, and will have clinical significance for a wide range of skin disorders where Treg cell-based therapies offer new therapeutic strategies.

(1:42pm)

EVIDENCE FOR THE EXPRESSION OF TYPE 1 INTERFERONS IN SCALP LESIONS OF ALOPECIAL AREATA

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Dept of Dermatology & Skin Science, University of British Columbia, Canada.

The exact mechanisms involved in the genesis of alopecia areata (AA) remain unknown. T lymphocytes play an essential role in the hair follicle (HF) injury that occurs in AA. Type 1 interferons (IFNs) enhance a Th1 immune response and recruit T-cells expressing $CXCR3^+$. Little is known about the role of Type1 IFNs and $CXCR3^+$ cells in lesions of AA. We examined the expression of IFN⊙ inducible myxovirus protein A (MxA) and $CXCR3^+$ T cells in paraffin embedded sections of scalp lesions from total of 8 patients with AA and compared those with lesions from 2 discoid lupus erythematosus (DLE), 2 lichen planopilaris (LPP) and 2 androgenic alopecia (androgenic A). We observed strong

expression of MXA in follicular epithelial cells and epidermal keratinocytes and appearance of $CXCR3^+$ cells surrounding the bulbar regions of HF in early AA similar to lesions from DLE and LPP. In contrast, chronic (non-inflammatory) lesions of AA and lesions of androgenic A failed to show the expression of MXA or CXCR3. Granzyme B (GrB) and T-cell restricted intracellular Ag1 (TiA-1) are both cytotoxic granule proteins and induce apoptosis and tissue damage. Lesions of AA showed expression of (TiA-1) but not GrB. In contrast, lesions of scarring alopecia (DLE and LPP) demonstrated expression of both TiA-1 and GrB cytotoxic molecules. Thus type 1 IFN-mediated induction of Th1 inflammatory immune responses and recruitment of $CXCR3^+$ T cells may play role in early lesions of AA. Furthermore, cytotoxic cells in AA lesions are functionally different than in DLE or LPP.

(1:54pm)

STROMAL CELL EXPRESSION OF *TOLL-LIKE RECEPTOR 9* IS REQUIRED FOR THE TOPICAL ADJUVANT EFFECT OF CPG OLIGODEOXYNUCLEOTIDES

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

Current vaccine technologies remain inadequate to counter the risk of pandemics of viral infections. This is in part due to the poor ability to induce effective antiviral immunity mediated by cytotoxic T lymphocytes (CTLs). We have demonstrated that topical administration of immunostimulatory CpG oligodeoxynucleotide (ODN), a Toll-like receptor 9 (TLR9) agonist, induces potent, rapid and durable antigen (Agn)-specific CTL responses to locally injected protein Agn. However, the subset of cells that are responding to this adjuvant effect remains to be elucidated and the expression of TLR9 in the skin is controversial. This study aimed to determine the expression of TLR9 by real time-PCR in the skin and if TLR9 is required on stromal cells such as keratinocytes for the topical adjuvant effect of CpG ODN *in vivo* using C57BL/6 mice. Bone-barrow chimeric mice with TLR9 deficient stromal cells but TLR9 sufficient (WT) bone marrow (BM)-derived cells were generated. Real time-PCR results indicated that untreated WT mice expressed TLR9 compared to TLR9 deficient mice in the skin. Tape stripping did not up-regulate TLR9 expression in WT mice while CpG adjuvant up-regulated TLR9 expression in the skin. Topical CpG treatment enhanced Agn-specific CTL responses in WT (TLR9 sufficient) but not stromal cell TLR9 deficient mice as indicated by the percent of OVA-specific CTLs amongst CD8+ T cells in the spleen. Thus, skin stromal cell expression of TLR9 is required for the topical adjuvant effect of CpG to increase the efficacy of vaccines.

(2:06pm)

LOSS OF INTEGRIN αVβ6 CAUSES ENHANCED KERATINOCYTE PROLIFERATION AND RETARDED HAIR FOLLICLE REGRESSION IN VIVO

Yanshuang Xie[1], Kevin McElwee [2], Lari Häkkinen[1] and Hannu Larjava[1]
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Integrin αvβ6 is an epithelial-specific receptor that is induced during wound healing. β6 integrin knock-out (β6-/-) mice exhibit an accelerated wound repair compared to wild-type (WT) mice and show increased number of proliferating hair follicle keratinocytes in a compromised wound healing model suggesting that αvβ6 integrin may regulate hair follicle regeneration. To test this hypothesis, we investigated the function of αvβ6 integrin in keratinocytes during hair follicle regeneration. A standardized mouse model of depilation-induced hair cycling was established in both WT and β6-/- mice. The hair cycle stages were assessed by histology. The expression of αvβ6 integrin was studied in regenerating hair follicles by immunohistochemistry. Catagen development was compared in the WT and β6-/- mice by quantitative histomorphometry. Keratinocyte proliferation was assessed by Ki67 immunostaining. The abundance of αvβ6 integrin was strongly up-regulated during the hair follicle regeneration and was hair cycle-dependent. The strongest immunostaining of αvβ6 integrin was noted during the onset of hair follicle involution. At day 20 after depilation, hair follicles in β6-/- mice were still in the early catagen, whereas hair follicles of WT mice had already entered late catagen and the subsequent telogen phase, suggesting that deletion of αvβ6 integrin causes retardation of hair follicle regression. β6-/- mice displayed more Ki67-positive cells than comparable catagen follicles from WT mice. These data suggest that αvβ6 integrin suppresses keratinocyte proliferation and induces hair follicle regression *in vivo.* Clinical Significance and KT: Suppressing αvβ6 integrin expression may provide useful therapeutic tools for human hair growth disorders.

(2:18pm)

IDENTIFICATION OF PATHOGENETIC GENE CHANGES IN SEZARY SYNDROME

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Sezary syndrome (SS) is an aggressive leukemic form of cutaneous T cell lymphoma, which is characterized by the hallmark Sezary cells with cerebriform nucleus in peripheral blood. To date, the molecular basis for the unique nuclear architecture and its link to Sezary cell behavioral features have not been elucidated. To identify pathogenic gene expression changes in Sezary cells, CD4+ T cells from peripheral blood of 6 SS patients, 9 healthy donors, 2 Sezary cell lines and 1 non Sezary T cell line were screened by Whole Human Genome Oligo Microarrays for differential gene expression profiles. 191 genes were found to be aberrantly expressed in Sezary cells. Those genes are involved in inflammatory response, cleavage of cytoskeletal proteins, dissembly of cell structures, and other biological functions which can be related to the malignant transformation of Sezary cells. Interestingly, we found that special AT rich binding protein 1(SATB1), which is a chromatin organizer in nucleus, was consistently and specifically down regulated in Sezary cells. Western blot and immunofluorescence validated its low expression in Sezary cell nucleus. As a global gene organizer involved in multiple cancers, SATB1 may contribute to the altered nuclear structure and the development of many of the gene expression changes observed in the Sezary cells. Clinical significance and knowledge translation: This pilot study may lead to new knowledge on the pathogenesis of Sezary syndrome and potentially point to new directions for the development of selective therapies for Sezary syndrome, which currently does not have a cure.

(2:30pm)

KERATINOCYTES COMMUNICATE WITH FIBROBLASTS RELEASING ANTI-FIBROGENIC FACTORS

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Introduction: Keratinocytes undergo continuous self-renewal. Through proliferation in the basal layer to a process of terminal differentiation, finishing with an impermeable keratinized layer. Ca^{2+} plays an important role in this process. Previously we demonstrated that stratifin, is externalized through exosomes and possesses a strong collagenase (MMP-1) stimulatory effect in fibroblasts. We hypothesize that keratinocytes might also release other proteins through exosomal externalisation whose profile would be different between differentiated (DK) and undifferentiated keratinocytes (UK). Results: Results revealed that stratifin is highly expressed in DK compared to the UK. DK and UK conditioned media stimulate MMP-1 in dermal fibroblasts, but DK one stimulate twice as much as UK. Proteomic analysis and western blot showed that exosomes from UK contain four out of seven 14-3-3 protein isoforms (β, η, ζ and γ) whereas exosomes from DK contain all seven 14-3-3 isoforms (σ, β, η, ε, ζ, γ and τ). We also showed that not only stratifin is responsible for MMP-1 stimulation in fibroblasts; 14-3-3

β also stimulates it. We also demonstrated that Ca^{2+} increases the release of exosomes 4.2 times more relative to control. Conclusions: In conclusion, exosomes from DK possess a different 14-3-3 protein profile than UK. In addition to stratifin, other 14-3-3 isoforms also stimulate MMP-1 expression. Clinical Significance: Exosomes have the potential to be used as a delivery mechanism. This study shows that keratinocyte exosomes contain anti-fibrogenic factors, with the capacity to induce MMP-1 expression in fibroblasts. The presence of these anti-fibrogenic factors in exosomes might be the answer to reduce scar tissue formation and improve hypertrophic scar.

(2:42pm)

MECHANISMS UNDERLYING SELECTIVE RESISTANCE OF SKIN CELLS VERSUS IMMUNE CELLS IN IDO-INDUCED TRYPTOPHAN DEFICIENT ENVIRONMENT

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Introduction: We have previously demonstrated that indoleamine 2, 3-dioxygenase (IDO), a tryptophan-degrading enzyme, causes apoptosis in immune, but not skin cells and showed that the stress-response-kinase GCN2 is involved in this selective apoptotic effect. However, the mechanism(s) underlying selective GCN2 pathway activation in immune cells is not known. It is showed that the protein IMPACT homolog inhibits GCN2 activation and abolishes the expression of its downstream target gene CHOP. Here, we asked the question of whether the expression of IMPACT protein in immune and skin cells is differently regulated in response to IDO-induced tryptophan deficient environment. Materials and Methods: IFN-γ treated IDO-expressing fibroblasts were co-cultured with bystander human $CD3^+$ Tcells, Jurkat cells, fibroblasts or keratinocytes. Expression of IMPACT was studied by Western blot and RT-PCR. The levels of total GCN2, phospho-GCN2 and CHOP were also studied by Western blot analysis. Results: A significant expression of phospho-GCN2 and CHOP was shown in immune cells, but not in skin cells, co-cultured with IDO-expressing fibroblasts. The IMPACT protein was highly and constitutively expressed in skin cells while its level was very low in $CD3^+$ Tcells and it was undetectable in Jurkat cells. IMPACT expression level in these cells was not dependent on either rich or amino acid-deprived environment. Conclusion: High expression of IMPACT in non-immune cells might act 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.

(2:54pm)

FORMULATION AND TOPICAL APPLICATION OF AN ANTI-FIBROGENIC GEL TO IMPROVE HYPERTROPHIC SCARRING

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Introduction: Hypertrophic scarring is an important clinical problem that lacks definitive treatment and results in disfiguring and functional impairment. It was shown by our group that stratifin stimulates the collagenase (MMP-1) expression in dermal fibroblasts *in vitro*. The goal of this study was to make a topical anti-fibrogenic gel containing stratifin and examine its therapeutic effect *in vivo* on the established fibrotic rabbit model. Methods: Sodium carboxymethyl cellulose (CMC) gel containing 0.2% stratifin was prepared and applied topically twice 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 hypertrophic scaring formation was evaluated. Results: Qualitative and quantitative wound assessments showed a significantly reduced scar hypertrophy in stratifin-treated wounds compared to controls. Wounds treated with stratifin-containing CMC gels demonstrated 80% decrease in scar hypertrophy compared to controls. Interestingly, a 2.8-fold ($p<0.001$) increase in MMP-1 expression and 90% ($p<0.001$) decrease in collagen density were observed in stratifin-treated wounds versus controls. Discussion: In this clinically relevant rabbit model, wounds treated with stratifin-containing CMC gels demonstrated a significant decrease in scar hypertrophy. These observations may be the result of increased breakdown of extracellular matrix components such as type-I collagen. Clinical Significance and KT: The findings of this study will ultimately be helpful in development of novel preventive strategies for hypertrophic scarring which are cost effective and feasible for all burn patients and particularly for children who are more vulnerable to multiple painful steroid injections or tight pressure garments.