Skin Research Group *Of* Canada

7th Annual Virtual Conference

PROGRAM & ABSTRACTS

November 12 – 13, 2020

Sponsors

Skin Research Group *of* Canada 2020

The SRGC Conference Scientific Organizing Committee is delighted to invite you to the 2020 Virtual Meeting, which will undoubtedly help bring together Canadian basic science, translational and clinical skin research expertise.

Our meeting will be equally beneficial to clinicians, basic scientists and industry partners engaged in skin disease research and will showcase world-class studies on psoriasis, eczema, skin cancer, autoimmune diseases, wound healing, skin regeneration and many other topics.

The focus of the meeting is to promote translational research within our research community. This meeting will help build critical connections among basic researchers, patient alliance/patient advocacy groups, clinicians, and industry partners to address critical questions to facilitate diagnosis and management of skin diseases.

Our invited keynote speakers will provide state-of-the-art lectures on major aspects of skin biology. Dr. Michael Underhill, (University of British Columbia) and Dr. Jeff Biernaskie, (University of Calgary) will discuss the contribution of mesenchymal progenitor cells in tissue repair, while Dr. Pierre Coulombe (University of Michigan Medical School) will present data describing the role of keratins in skin homeostasis, wound healing and disease and Dr. Edward Tredget (University of Alberta) will outline novel strategies to treat hypertrophic scars in burn victims.

Make your plans to join us virtually in this celebration of Canadian skin research.

We need your participation to make this meeting a success!

Sincerely,

SRGC2020 Organizing Committee Co-chairs

Dr. Andrew Leask
University of Saskatchewan

Dr Aziz Ghahary
University of British Columbia

SRGC 2020 Virtual Program

THURSDAY, 12 NOVEMBER		
1:00 - 1:15pm		Welcome Remarks by SRGC President Anie Philip Opening Remarks by Co-chairs Andrew Leask/ Aziz Ghahary
1:15-1:45pm		***SRGC State-of-the-art Lecture*** **Dr. Ted Tredget** **University of Alberta**
1:45 – 3:00pm	**Session I: Wound Healing, Inflammation and Fibrosis** **Moderators: Aziz Ghahary/Amani Hassan**	
	1:45pm	**Veronique Moulin, University Laval** Role of PlGF-1 contained in cutaneous myofibroblast-derived microvesicles in the production of extracellular matrix
	2:00pm	**Christopher Turner, University of British Columbia** Granzyme B: A Key Mediator of Age-Impaired Wound Healing Caused by Pressure Injury
	2:10pm	**Katlyn Richardson, University of British Columbia** Granzyme K Is A Potential Therapeutic Target for Aging Skin
	2:20pm	**Brian Wu, University Health Network** EphB4 Signaling in The Development of Pulmonary Fibrosis
	2:30pm	**Maha Alsharqi, McGill University** Regulation of Pulmonary Fibrosis By CD109 In A Murine Mode
	2:40pm	**Antoinette Nguyen, University of Alberta** The Identification of Monocyte Subpopulations and Macrophage Phenotypes During Wounding Healing in the Dermal Fibrotic Mouse Model
	2:45pm	**Lindy Schaffrick , University of Alberta** Chemokine Response During Wound Healing After Burn Injured Patients
	2:50pm	**Meryem Safoine, LOEX** Enhanced Diabetic Wound Healing Using Adipose-Derived Stromal Cell-Based Biological Dressings Produced Under Serum-Free Conditions
	2:55pm	**Holly Sparks, University of Calgary** A peptide-modified hydrogel improves wound healing in a human to mouse xenograft model
3:00 - 3:15pm		Poster Session Display I
3:15-3:45pm		***SRGC Excellence in Skin Research Lecture*** "Mesenchymal ("stem cells") progenitors in health and disease" **Dr. Michael Underhill** **University of British Columbia**

SRGC 2020 Virtual Program

3:45 – 5:00pm	**Session II: Microenvironment** **Moderators: Lucie Germain/Meryem Safoine**	
	3:45pm	**Andrew Leask, University of Saskatchewan** Insights into Fibroblast Plasticity: Cellular Communication Network 2 Is Required for Activation of Cancer-Associated Fibroblasts in A Murine Model of Melanoma
	4:00pm	**Hana Hakami, McGill University** Fibulin-4 And Latent Transforming Growth Factor-B Binding Protein-4 Cell Interactions in Elastogenesis
	4:15pm	**Mathias Lemarchand, LOEX** Substance P, CGRP And Aminoguanidine Improve Re-Epithelialization In Vitro In A Glycated Tissue-Engineered Wound Healing Model
	4:25pm	**Rong-Mo Zhang, McGill University** Fibrillin-1 And Fibronectin RGD Motifs Post-Transcriptionally Regulate ERK1/2 Signaling and Fibroblast Proliferation Via miR-1208
	4:35pm	**Valentin Nelea, McGill University** Role of N-linked glycans in Fibulin-5 and LTBP-4S mediated matrix assembly and function
	4:40pm	**Qianli Yang, University of British Columbia** Non-Melanocytic Cellular Changes in Vitiligo Skin Microenvironment and Their Correlation with Clinical Phenotype and Response to Therapy
	4:45pm	**Shivshankari Rajkumar, McGill University** Combination BRAF And MEK Inhibition Is Effective in The Treatment of BRAF Non-P. V600 Mutant Melanomas with Co-Occurring NF1 Loss-Of-Function or Oncogenic NRAS Alterations
	4:50pm	**Gaurav Isola, McGill University** Staphylococcus Aureus Proteoglycans Can Limit Type 2 T Cell Differentiation: Implications for Atopic Dermatitis
4:55pm		**Closing remarks of Day 1**
END OF THE DAY 1		
FRIDAY, 13 NOVEMBER		
1:00 – 1:15pm	**Opening Remarks Day 2**	
1:15-1:45pm		
	SRGC State-of-the-art Lecture	
	Dr. Jeff Biernaskie **University of Calgary**	
1:45 – 3:00pm	**Session III: Novel analytical approaches** **Moderators: Michael Underhill/ Katlyn Richardson**	
	1:45pm	**Amani Hassan, McGill University** CD109 Activates EGFR/STAT3 Signaling in Squamous Cell Carcinoma

	2:00pm	**Carolyn Jack, McGill University** Patient-Centered Development of a Mobile Health Application for Integrated Knowledge Translation in Adult Atopic Dermatitis
	2:15pm	**Vladimir Andrey Gimenez Rivera, McGill University** Identifying the Cutaneous Molecular Signature of Chronic Inflammation in Atopic Dermatitis: Approaching personalized medicine through proteomics
	2:25pm	**Anastasiya Muntyanu, McGill University** Geographic Clustering of Systemic Sclerosis in Canada And Study of Potential Environmental Triggers
	2:35pm	**Brice Magne, LOEX** Development of A Skin Pigmentation Model for The Study of Melanocytes and Photobiology
	2:40pm	**Garima Kulshreshtha, University of Ottawa** Eggshell Membranes: A Collagenous Biomaterial for Cosmetics and Skin Health Applications
	2:45pm	**Manar H. Younes, University of Ottawa** Use of Seaweeds in Cosmetics and Skin-Care Applications
	2:50pm	**Ling Wu, University of Ottawa** Particalized Eggshell Membrane (PEM) For Cosmetics and Biomedical Applications
	2:55pm	**Emilie Attiogbe, LOEX** A New 3D Vascularized Skin Model Containing Immune Cells Reconstructed by Tissue Engineering
3:00- 3:15pm		Poster Session Display II
3:15-3:45pm		
		SRGC Frontiers in Science Lecture
		Dr. Pierre A. Coulombe **University of Michigan Medical School**
3:45 – 4:45pm		**Session IV: Clinical and therapeutic considerations** **Moderators: Julie Fradette/ Brice V Magne**
	3:45pm	**Luciola Silva Barcelos, Université Laval** Effects of Sodium Butyrate on Vascularisation and Matrix Remodelling in A Model of Granulation Tissue in Mice - Involvement of The Receptor Gpr43
	4:00pm	**Aishwarya Iyer, University of Alberta** Assessment of T-Cell Repertoire in Non-Hodgkin's T-Cell Lymphomas
	4:05pm	**Ilya Mukovozov, University of British Columbia** Preliminary Results from The Hidradenitis Suppurativa Patient Experience (Hspe) Survey
	4:10pm	**Raymond Milan, McGill University** Sex Differences in Patterns of Systemic Agents' Use Among Individuals with Psoriasis
	4:15pm	**Aaron Drucker, University of Toronto** Antihypertensive Medication Use and Risk of Skin Cancer

	4:30pm	**Jennifer Gantchev, RI-McGill University** The Relationship Between Meiomitosis, The meictGene HORMAD1 And Genomic Instability in Head and Neck Squamous Cell Carcinomas
	4:35pm	**Philip Surmanowicz, University of Alberta** Primary Cutaneous CD4+ Small/Medium-Sized Pleomorphic T-Cell Lymphoproliferative Disorder: The Alberta Perspective and Review of The Literature
4:40pm		**SRGC 2021 announcement by Dr. Ivan V Litvinov**
4:45pm		**Closing Remarks - Awards will be posted on the website**
5:00pm		*Board Meeting*

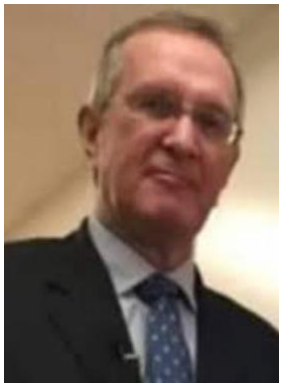
Dr. TED TREDGET

University of Alberta

November 12, 2020

SRGC STATE-OF-THE-ART LECTURE

"Wound Healing in the Thermally Injured Patient: Can We Control the Scarring?"



Dr. Edward (Ted) Tredget received his medical degree with distinction from the University of Alberta, Canada in 1976 and went on to complete his internship, general surgery and plastic surgery training from the University of Alberta in 1984. He undertook a Post-Doctoral Fellowship at the MIT Massachusetts General Hospital and Harvard Medical School in Boston from 1984 to 1987 where he received his Masters of Science degree in Applied Biological Sciences. He was awarded the American Burn Association's Travelling Fellowship in 1988. Upon his return to the University of Alberta in 1988, Dr. Tredget became Director of the Firefighters' Burn Treatment Unit and the Plastic Surgery Wound Healing Research Laboratory of the University of Alberta. The focus of their research includes basic science research relating to nosocomial infections, wound healing, hypertrophic scarring and gene therapy. They have several ongoing clinical trials and research projects relating to metabolism following burn injury, rehabilitation of the burn patient, wound healing and the potential role of gene therapy for the management of post burn hypertrophic scarring. Dr. Tredget is currently a Professor in the Department of Surgery, Divisions of Plastic & Reconstructive Surgery and Critical Care Medicine at the University of Alberta. Dr. Tredget is the Past President (2015-16) of the American Burn Association and the Canadian Society of Plastic Surgeons (2014-15).

Dr. MICHAEL UNDERHILL

University of British Columbia

November 12, 2020

SRGC EXCELLENCE IN SKIN RESEARCH LECTURE

"Mesenchymal ("Stem Cells") Progenitors in Health and Disease"

T. Michael Underhill is a Professor in the Department of Cellular and Physiological Sciences at the University of British Columbia (UBC). He completed his Ph.D. at Western University (1991) and carried out post-doctoral training at Duke University (1991-95) where he studied the function of the retinoid signaling in limb development. Following this, he became an Assistant Professor at Western University (1996) where his group described fundamental roles for retinoid signaling in regulating mesenchymal cell fate in skeletogenesis. In 2004, he joined the University of British Columbia and his interests expanded to include investigating how mesenchymal progenitors (MPs) support tissue renewal and regeneration, and the role of MPs in tumorigenesis.

Dr. JEFF BIERNASKIE

University of Calgary

November 13, 2020

SRGC STATE-OF-THE-ART LECTURE

Dr. Jeff Biernaskie completed his BSc in Neuroscience at the University of Lethbridge, his PhD in Neuroscience at Memorial University and postdoctoral training in Stem Cell Biology at the Hospital for Sick Children in Toronto. In 2009, he joined the Faculty of Veterinary Medicine at the University of Calgary, where is he currently a Professor in Stem Cell Biology and Regenerative Medicine. He is a member of the Alberta Children's Hospital Research Institute and the Hotchkiss Brain Institute and he holds the Calgary Firefighters Burn Treatment Society Chair in Skin Regeneration and Wound Healing. His research program is focused on understanding the cellular and molecular mechanisms underlying tissue regeneration and the potential to exploit tissue-resident stem cells toward restoration of function following injury or disease. He is specifically interested in how skin and brain stem/ progenitors cells are regulated during homeostasis and how factors like injury, advanced aging and the immune system impact their function.

Dr. PIERRE A. COULOMBE

University of Michigan Medical School

November 13, 2020

SRGC FRONTIERS IN SCIENCE LECTURE

Dr. Pierre A. Coulombe, a native of Montréal, Québec, Canada, serves as the G. Carl Huber Professor and Chair of the Department of Cell & Developmental Biology at the University of Michigan Medical School. He is jointly appointed in the Department of Dermatology and is a member of the Comprehensive Cancer Center at the same institution. Dr. Coulombe joined U-M in 2017 following a 25 year-stay on faculty, including 9 years as department chair, at the Johns Hopkins University. Dr. Coulombe received a Ph.D. degree in Pharmacology from the Université de Montréal and pursued postdoctoral training at the University of Chicago. Dr. Coulombe played a key role in defining the vital role of mechanical support fulfilled by keratin filaments in epithelial cells and tissues, in the identification of the first intermediate filament-based disease, and in the discovery of several novel ("non-canonical") roles for keratin proteins. In addition to its focus on the pathophysiology of rare monogenic skin disorders, research in the Coulombe laboratory in recent years has been increasingly focused on the basic biology of cancer and on the significance of the newly found presence of keratin proteins within the nucleus. Throughout his career Dr. Coulombe has been very active in the education and training of graduate students and postdoctoral fellows, and in the recruitment and mentoring of junior level faculty.

Session I: Wound Healing, Inflammation and Fibrosis

ROLE OF PLGF-1 CONTAINED IN CUTANEOUS MYOFIBROBLAST-DERIVED MICROVESICLES IN THE PRODUCTION OF EXTRACELLULAR MATRIX

Véronique J. Moulin, Syrine Arif, Sébastien Larochelle, Jason Dagher
University Laval

We have previously demonstrated that normal skin wounds myofibroblasts (Wmyo) can communicate with other cells using secreted factors but also using microvesicles (MVs), a type of extracellular vesicles that contained mediators of intercellular communication.

We have evaluated the effect of Wmyo-derived MVs on dermal fibroblasts and determine the signaling molecule responsible to the effects on cells. MVs were obtained from culture media of myofibroblasts and characterized using protein quantification, dynamic light scattering, transmission electron microscopy and a multiplex ELISA.

Following the treatments of skin fibroblasts with different concentration of MVs or a selected cytokine, parameters linked to the extracellular matrix were studied.

First, we showed that fluorescent Wmyo derived-MVs were internalized by dermal fibroblasts. Cytokine array analysis revealed that the cytokine that was in the higher amount in MVs was placental growth factor (PlGF) (0.88 ± 0.63 pg/µg proteins in MVs). Treatments with MVs or PLGF-1, but not PlGF-2, induced a significantly increase of procollagen I level production (Fold change of 1.80 ± 0.18 and 2.07 ± 0.18, respectively) whereas the neutralization of PLGF-1 in MVs significantly inhibited the production of procollagen I by fibroblasts.

Our study shows that extracellular matrix production can be modulated by Wmyo derived-MVs through PLGF-1 signalling during wound healing.

GRANZYME B: A KEY MEDIATOR OF AGE-IMPAIRED WOUND HEALING CAUSED BY PRESSURE INJURY

Christopher T Turner 1,2, Matthew R Zeglinski 1,2, Juliana Bolsoni 1, Hongyan Zhao 1,2, Anthony Papp 1,2, David J Granville 1.

1. Department of Pathology and Laboratory Medicine, UBC, Vancouver, BC, Canada

2. International Collaboration on Repair Discoveries (ICORD) Centre, Vancouver, BC, Canada

Introduction

Pressure injuries (PI), also known as pressure ulcers or bedsores, are on the rise due to our progressively aging and sedentary population. While aging is a major risk factor for PIs, their study in murine models of skin aging are limited. Apolipoprotein E knockout (ApoE-/-) mice are atherosclerotic and exhibit accelerated skin aging and impaired wound healing. Granzyme B (GzmB) is a serine protease minimally expressed in normal skin but dramatically elevated in several age-related chronic inflammatory skin diseases and contributes to impaired diabetic wound healing. We hypothesized GzmB contributes to the development, severity and impaired healing of PI's.

Methods

To evaluate GzmB expression, human PI wound fluid (ELISA) and excised tissue (histology) was collected and examined. A causative role for GzmB was assessed in GzmB-/- and WT mice through the induction of PI via multiple rounds of ischemia-reperfusion injury. Additionally, PI's were induced in ApoE-/- and GzmB/ApoE double knockout (DKO) mice fed on a high fat diet for 30 weeks, which allowed an aged phenotype reflective of the key population demographic for PI. Wound morphometry, skin tensile strength, extracellular matrix (ECM) remodeling, fibrosis, inflammation, tissue perfusion and vascular hemosiderin extravasation were assessed.

Results

GzmB was dramatically elevated in PI patient wound fluid and excised tissue, and positively correlated to disease severity. There was no difference in PI severity between GzmB-/- and WT mice. However, in the aged mice, PI severity was significantly reduced in DKO compared to ApoE-/- mice, involving improved wound closure, increased tensile strength, improved ECM remodeling in response to GzmB-mediated decorin cleavage, reduced fibrosis, and overall inflammation reduction. Reduced IL-16, IL-1β, TIMP-1 and TREM-1 expression was identified in DKO mice. sICAM, a marker of vascular wall inflammation, was also reduced in DKO tissue extract, with this associated with a decrease in the leakage of blood from vessels and into the wound area.

Conclusions

GzmB is elevated in PI and contributes to increased wound severity, thus may provide a promising therapeutic target.

Learning objectives

1. Granzyme B is emerging as an important mediator of skin injury, inflammation and repair.
2. Granzyme B impairs the healing of pressure injuries in aged skin.
3. Inhibition of granzyme B may provide a therapeutic option for the treatment of wounds in an aging population.

Takeaway Message

Granzyme B is dramatically elevated in severe pressure injury, and contributes to impaired wound repair, functioning through increased ECM degradation, elevated microvascular hemorrhage and the induction of a fibrotic phenotype.

GRANZYME K IS A POTENTIAL THERAPEUTIC TARGET FOR AGING SKIN

Katlyn Richardson(1,2); Matthew Zeglinski(1,2), Nick J. Carr(3), David J. Granville(1,2,4)

(1) International Collaboration On Repair Discoveries (ICORD), Vancouver Coastal Health Research Institute, University of British Columbia, Vancouver, Canada; (2) Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada; (3) Department of Surgery, University of British Columbia, Vancouver, Canada; (4) British Columbia Professional Firefighters' Burn and Wound Healing Group, Vancouver, Canada.

Introduction

Intrinsic skin aging is an inevitable phenomenon characterized by the phenotypic modulation and eventual involution of dermal white adipose tissue (dWAT), leading to impaired skin immune function and increased susceptibility to injury. Unfortunately, current therapies are limited. As such, a deeper understanding of the mechanisms associated with dWAT in intrinsic skin aging is necessary. Granzyme K (GzmK) is a serine protease recently elucidated as a mediator of cutaneous inflammation. Our lab has observed GzmK to be implicated in altered cell volume of dWAT. However, the role of GzmK in intrinsic skin aging is unknown. In the present study, we hypothesize that GzmK contributes to impaired skin immune function and integrity in intrinsically-aged skin by altering the morphology of adipocytes.

Methods

GzmK expression was evaluated histologically in human abdominal skin from age-matched young (~40 years) and old (~70 years) individuals. The role of GzmK was investigated using apolipoprotein E knockout (ApoE-/-) mice, an established model of accelerated skin aging, comparing Wild type (WT), ApoE-/-, GzmK-/-, and ApoE-/-GzmK-/- double knockout (DKO) mice. Skin aging severity was assessed macroscopically. Skin tissue was examined histologically for epidermal thickness and inflammatory cell infiltrate. To elucidate a mechanistic role, we will culture adipocytes with GzmK for assessment of size and morphology, cytokine expression and to define the GzmK degradome.

Results

There was a significantly greater number of GzmK positive cells in aged human skin compared to young, healthy skin. Preliminary evidence shows DKO mice exhibit an increase in epidermal thickness and tensile strength compared to APOE-/-, GzmK-/- and WT mice. DKO mice also exhibit an increase in adipocyte cell volume and thickness of dWAT layer compared to APOE-/-, GzmK-/- and WT mice.

Conclusions

GzmK is elevated in aged human skin and may contribute to physiologic aging of skin by altering the morphology (specifically, cell volume and thickness) of dWAT.

Learning objectives

1. GzmK promotes inflammation in the skin;
2. GzmK is elevated in aged human skin; and
3. GzmK-induced inflammation may contribute to intrinsic-skin aging by altering the morphology of dWAT

Takeaway Message

GzmK is elevated in aged human skin and may contribute to physiologic aging of skin by altering the morphology of dWAT. This study could provide the first evidence for GzmK as a therapeutic target for intrinsically-aged skin.

EPHB4 SIGNALING IN THE DEVELOPMENT OF PULMONARY FIBROSIS

Wu B, Tang L, Nakamura S, Rossomacha E, Monnier P, Wu J, Hinz B, Lagares D and Kapoor M.

1. University Health Network, Toronto, Canada.

2. University of Toronto, Toronto, Canada.

3. University of Montreal Hospital Research Centre, Montreal, Canada.

4. Harvard Medical School, Boston, USA.

Introduction

Fibrosis can cause the loss of tissue function, organ failure and death through excessive deposition of extracellular matrix. Currently there is unclear etiology and limited treatment for complete pathology resolution. We recently identified fibroblast-derived ephrin B2 as a pro-fibrotic mediator and Efnb2 knockout (KO) in fibroblast yielded partial protection against the development of fibrosis in lung and skin. We have found that the partially protective phenotype observed can be recapitulated by endothelial knockout of ephrin B2, suggesting that cellular source of ephrin B2 may not be a strong contributor to disease development. We have recently found that fibroblast knockout of the ephrin B2 receptor, ephB4, garners significant protection against the development of pulmonary fibrosis.

Methods

To understand the role of fibroblast-derived ephB4 in fibrosis, we generated Col1Î±1-Cre Ephb4 KO mice and subjected them to bleomycin-induced lung fibrosis. Lungs collected from these mice 14-days post bleomycin were evaluated through histology to assess fibrosis development. Fibroblast were also cultured from mouse lungs and subjected to western blotting, RT-qPCR and proliferation assessment.

Results

We have found that ablation of fibroblast-Ephb4 KO is protective against fibrosis development. Proliferation decreases in response with Ephb4 knockout in fibroblasts as well as decrease in pro-fibrotic markers.

Conclusions

Current therapies for fibrosis are broad targeting and do not completely resolve disease activity. The understanding of ephrin B2 interaction with ephB4 as a potential disease mediating signaling pathway may uncover novel and specific therapeutic targets.

Learning objectives

1. Ephrin B2 signaling from either fibroblasts or endothelial cells contribute to pulmonary fibrosis.
2. EphB4 is a potential signaling partner to ephrin B2 on fibroblasts in the lung and contribute to pulmonary fibrosis.
3. EphB4 signaling appears to influence fibroblast proliferation and myofibroblast transition.

Takeaway Message

Ephrin B2 and EphB4 interaction remains unclear in the development of pulmonary fibrosis but may act as novel therapeutic targets for either treatment or prevention.

REGULATION OF PULMONARY FIBROSIS BY CD109 IN A MURINE MODEL

Maha Alsharqi 1, Yumiko Ishii 2 , Meryem Blati 1, James Martin 2 and Anie Philip 1

1Division of Plastics Surgery, McGill University, Montreal General Hospital

2Department of Medicine, McGill University and the Research Institute of the McGill University Health Centre

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease characterized by inflammation, excessive fibroblasts proliferation, differentiation, and deposition of extracellular matrix (ECM) in the interstitium. It will lead to impairment in the quality of life and decline in lung functions. Transforming Growth Factor-ß1 (TGF-ß1) is a multifunctional growth factor, with a wide range of functions in homeostasis and tissue repair. TGF- ß1 signaling is mediated via Smad2/3 intracellular proteins leading to gene transcription and ECM protein expression.

TGF- ß1/Smad2,3 signaling pathway plays an important role the pathogenesis of lung fibrosis and many other fibrotic disorders. Our team has previously identified CD109 a glycosylphosphatidylinositol (GPI)-anchored protein as a TGF-ß1 co-receptor that negatively regulates TGF-ß1 signaling and that this involves caveolae-mediated internalization and receptor degradation. We have previously shown that CD109 deficient mice display enhanced TGF-ß1 signaling leading to an increased fibroblast proliferation and ECM deposition in a skin fibrosis model. In this study, we aim to investigate the role of CD109 on lung fibrosis using a CD109 deficient mouse model.

Methods

Cellular migration was analyzed using invitro wound healing assay. The architecture of the lung alveoli and collagen deposition were analyzed by hematoxylin and eosin staining, and Masson's trichrome staining, respectively. Immunohistochemistry was performed to evaluate the expression of different ECM markers. Finally, evaluated lung compliance using FlexiVent on CD109 deficient mice in comparison to WT mice.

Results

In vitro wound healing assay shows that CD109 KO lung fibroblasts display greater TGF-β induced migration than WT lung fibroblasts ($p<0.05$). Interestingly, H&E staining reveals that the KO lungs exhibit increased cellularity and distinct alveolar morphology that could be due to obliteration of the alveolar sacs, when compared to WT lungs. Trichrome staining demonstrates a markedly increased collagen content in CD109 KO lungs compared to WT lungs. Furthermore, the KO lung fibroblasts show increased expression of collagen, fibronectin and alpha smooth muscle actin, as detected by immunohistochemistry.

Conclusions

Our finding that CD109 deficiency results in markedly enhanced TGF-β signaling, fibrotic responses, and cellularity in the mouse lung signify the importance of CD109 in ECM synthesis and homeostasis of the lung in mice.

Learning objectives

1. Acquire knowledge about lung fibrosis.
2. Understand fibrosis and its link to the TGF-β signaling pathway.
3. Learn the mechanism of action of CD109 in regulating lung fibrosis.

Takeaway Message

CD109 has an important role in TFG-β signaling pathway and lung fibrosis.

THE IDENTIFICATION OF MONOCYTE SUBPOPULATIONS AND MACROPHAGE PHENOTYPES DURING WOUNDING HEALING IN THE DERMAL FIBROTIC MOUSE MODEL

Antoinette T. Nguyen, Jie Ding, Elcin Alpat, Hirokatsu Umeyama, Edward E. Tredget

Wound Healing Research Group, Department of Surgery, University of Alberta

Introduction

Hypertrophic scars result from injury to the deep dermal layers and aberrant wound healing. During the inflammatory phase, monocytes recruited to the wound site differentiate into macrophages. Different macrophage phenotypes are known to play different physiological and pathological roles. This study aims to identify the distribution of monocyte populations and macrophage phenotypes during wound healing and scar formation in the dermal fibrotic mouse model.

Methods

To create the dermal fibrotic model, split thickness human skin were grafted onto the dorsum of athymic nude mice. Blood, spleen and scar tissues were collected at day 0 (d0, n=3), 3, 7, 14, 30, 60, 90, and 180 (n=6), and stained for monocyte populations and M1, M2a, M2b, M2c, and M2d macrophages.

Results

Wound contraction was apparent on d7. Increased skin thickness peaked on d30 in both epidermis and dermis. An increase in dermal cellularity on d180 and vascularity on d14 was observed. On d30, a significant increase was seen in CD11b+/Ly6C++/CCR2+ monocytes; whereas CD11b/Ly6C-/CCR2- monocytes peaked on d60. In the hypodermis, M1 had an initial peak on d7 and a second peak between d30 and d60. In the upper dermis, M1 peaked on d60. An increase in M2b appeared in the hypodermis on d30. There were no changes in M2d numbers in the hypodermis, however, an increase in the upper dermis on d14 was detected.

Conclusions

During wound healing, the two waves of monocyte populations suggest early and late recruitment. Increased M1 may be associated with inflammation, proliferation and remodeling phases of wound healing. A rise in M2d coincided with increased vascularity on d14, implicating a role in angiogenesis. M2a/M2c analyses are underway. Identification of a pro-fibrotic monocyte/macrophage phenotype can be a potential intervention to prevent scarring.

Learning objectives

1. To characterize wound healing and scarring in a nude mouse model.
2. Reveal different monocyte populations and macrophage phenotypes present throughout wound healing and scarring.
3. Identify the potential pro-fibrotic monocyte/macrophage phenotype(s).

Takeaway Message

Monocytes/macrophages are key players in wound healing and scar formation. The objective of this research aims to identify a pro-fibrotic monocyte/macrophage phenotype that can be a potential therapeutic intervention to prevent dermal fibrosis.

CHEMOKINE RESPONSE DURING WOUND HEALING AFTER BURN INJURED PATIENTS

Lindy Schaffrick(1), Jie Ding(1), Peter Kwan(1), Edward Tredget(1, 2)

(1) Wound Healing Research Group, Division of Plastic and Reconstructive Surgery, Department of Surgery, University of Alberta, Edmonton, Alberta Canada

(2) Division of Critical Care, University of Alberta, Edmonton, Alberta Canada

Introduction

Burn injury hospitalizes 11 million people annually. Up to 70% of deep-dermal injury and prolonged inflammation will cause hypertrophic scars (HTS). HTS appear red, raised, and confine to the boundaries of the wound. HTS results in painful skin contraction restricting movement, as well as cosmetic stressors. HTS has no standard or reliable treatment outcome. Chemokine pathways play a critical role in inflammation through recruiting blood-borne cells into the wound site. In this study, we tracked the changes of chemokines and their receptor expressions in circulating monocytes during wound healing after burn injury.

Methods

Peripheral blood was collected from burn patients with varying total body surface area (TBSA) at 48 hours, 1 week, and 1 month after burn injury. Peripheral blood mononuclear cells were isolated and stained for CD14 and CD16 for monocytes, and CXCR4, CCR2, CCR5 for chemokine receptor expressions. Chemokines SDF-1, MCP-1, and RANTES were measured in the serum using cytokine biomarker assay.

Results

CD14++CD16+ monocytes are dominant in controls and burn patients, which are up-regulated more than CD14+CD16++ monocytes in burn patients. The expression of chemokine receptors (CXCR4, CCR2, CCR5) in CD14++CD16+ cells increased responding to burn injury and decreased over time in burns with > 20% TBSA compared to controls and burns with <20% TBSA. In burns, CXCR4 expression is lower at the earlier time-points, and shows higher expression over time. CCR5 expression also changed over time during wound healing. Data is still being analyzed.

Conclusions

Larger burns increase monocytes, especially the CD14++CD16+ population. Chemokine receptor expression of CXCR4 increases in CD14++CD16+ cells, and CCR5 shows different changes from patient to patient. Other data analysis will be completed prior to the meeting. We believe that the understanding of chemokine pathway signaling in burns will provide a targeted treatment for HTS in the future.

Learning objectives

To understand the behaviour of SDF-1/CXCR4, MCP-1/CCR2, and RANTES/CCR5 after burn injury, including chemokine levels in the serum and their receptor expressions on monocytes. Also, look assess total amount of TBSA in relation to the response of chemokines and their receptor expressions of monocytes.

Takeaway Message

Exploring the role between chemokines and scar development will help understand the mechanism of abnormal wound healing after burn injury. The goal is to identify a therapeutic target and highly effective treatment for clinical use in post burns and HTS.

ENHANCED DIABETIC WOUND HEALING USING ADIPOSE-DERIVED STROMAL CELL-BASED BIOLOGICAL DRESSINGS PRODUCED UNDER SERUM-FREE CONDITIONS

Safoine M 1, Paquette C 1, Fradette J 1,2

1. Centre de recherche en organogenèse expérimentale de l'Université Laval/LOEX, Québec

2. Department of Surgery, Faculty of Medicine, Université Laval, Québec

Introduction

Long term diabetes often leads to chronic wounds refractory to standard treatments. Cell-based therapies are actively investigated to enhance wound repair. Various cell types are used to produce biological dressings that can be applied on wounds such as adipose-derived stromal cells (ASC) which are an attractive cell source considering their abundancy and therapeutic properties.

Methods

In this study, we produced ASC-based dressings under a serum-free system using the self-assembly approach of tissue engineering. These dressings were applied to full thickness 8-mm splinted skin wounds created on the back of two diabetic murine models: polygenic diabetic NONcNZO10/LtJ mice and streptozotocin-induced diabetic K14-H2B-GFP mice displaying a fluorescent epidermis allowing tracking of reepithelization using an in vivo imaging system.

Results

NONcNZO/LtJ mice global wound closure kinetics evaluated by macroscopic imaging showed that ASC-based dressings accelerated wound closure by 83% at day 8, 57% at day 12 and 35% at day 16 compared to untreated wounds which consistently had a 1-week delay in healing. K14-H2B-GFP mice reepithelization kinetics showed no significant difference between treated and untreated mice despite the observed improvement in wound closure in treated wounds. On histological sections, treated wounds exhibited healed skin of better quality with a more organized, homogeneous and 1.6-fold thicker granulation tissue, and a well-differentiated epidermis. Neovascularization, assessed by CD31+ labeling, was 1.3-fold higher in the treated wounds.

Conclusions

We described the first entirely serum-free production system of naturally derived scaffold-free ASC-based biological dressings facilitating clinical translation. These tissue-engineered dressings represent promising candidates for diabetic cutaneous healing in vivo, by stimulating, among others, granulation tissue formation and neovascularization.

Learning objectives

1. Understand the importance of fetal bovine serum substitution in the production method of cell-based biological dressings considering clinical translation.
2. Discuss the impact of adipose-derived stromal cell-based biological dressings on global wound closure kinetics in diabetic mice.
3. Describe how adipose-derived stromal cell-based biological dressings impact granulation tissue formation, neovascularization and reepithelization.

Takeaway Message

We developed adipose-derived stromal cell-based biological dressings produced in a bovine serum-free setting compatible with clinical translation. These dressings accelerate wound closure by stimulating granulation tissue formation and neovascularization without significant impact on reepithelization kinetics.

A PEPTIDE-MODIFIED HYDROGEL IMPROVES WOUND HEALING IN A HUMAN TO MOUSE XENOGRAFT MODEL

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Introduction

Nonhealing wounds, which occur with increased frequency amongst the aged population as well as those with chronic disease, are a significant source of morbidity for patients. However, despite wound therapies being a multi-billion-dollar industry; safe and effective treatments aimed at restoring the skin's barrier function remain elusive. Here, we investigate the use of a proprietary peptide-conjugated hydrogel, previously shown to increase keratinocyte migration and improve wound healing in diabetic mice, on adult human skin.

Conclusions

Overall, this data provides further evidence that the use of the peptide-modified hydrogel in wounds will result in accelerated closure and improved outcomes for human patients.

Learning objectives

Here we aim to investigate if, in a xenograft model, the peptide-modified hydrogel shows promise for the treatment of wounds on human skin.

Takeaway Message

This proprietary peptide-modified hydrogel shows promise for improving the rate and quality of wounds on human skin through promotion of keratinocyte survival and migration rather than proliferation.

Session II: Microenvironment

INSIGHTS INTO FIBROBLAST PLASTICITY: CELLULAR COMMUNICATION NETWORK 2 IS REQUIRED FOR ACTIVATION OF CANCER-ASSOCIATED FIBROBLASTS IN A MURINE MODEL OF MELANOMA

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Introduction

Tumor stroma resembles a fibrotic microenvironment, being characterized by the presence of myofibroblast-like cancer-associated fibroblasts (CAFs). Tumor stroma is known to be negatively associated with the ability of patients to survive cancer; however, no drugs at present block the activity of the stroma in cancers. The matricellular protein CCN2 is overexpressed in cancers in tumor cells and the fibrotic microenvironment. CCN2 is a matricellular protein under clinical development for cancers, but the mechanism underlying CCN2 action is unclear.

Methods

We use a syngeneic model of melanoma metastasis and mice deleted for not for CCN2 in tumor cells or CAFs. CAFs were labeled postnatally with green fluorescent protein using mice expressing a tamoxifen-dependent Cre recombinase under the control of a fibroblast-specific promoter/enhancer.

Results

CCN2 expression by CAFs, but not tumor cells, was required for metastasis and neovascularization. Fibroblast activation was impaired in mice with a fibroblast-specific deletion of CCN2, associated with reduced expression of α-smooth muscle actin, periostin, integrin alpha 11 (ITGA11) and Sox2. Multipotent Sox2-expressing skin-derived precursor (SKP) spheroids were cultured from murine back skin. Using lineage tracing and flow cytometry, approximately 40% of SKPs were found to be derived from type I collagen-lineage cells and acquired multipotency in culture. Inhibition of mechanotransduction pathways prevented myofibroblast differentiation of SKPs and expression of Ccn2. In SKPs deleted for Ccn2, differentiation into a myofibroblast, but not an adipocyte or neuronal phenotype, was also impaired. In human melanoma, CCN2 expression was associated with a profibrotic integrin alpha (ITGA) 11-expressing subset of CAFs that negatively associated with survival.

Conclusions

These results suggest that synthetic dermal fibroblasts are plastic, and that CCN2 is required for the differentiation of dermal progenitor cells into a myofibroblast/CAF phenotype and, consequently, metastasis is, therefore, a therapeutic target in melanoma.

Learning objectives

1. CAFs are essential for melanoma metastasis
2. CAFs expressing CCN2 and ITGA11 are negatively associated with survival in melanoma
3. CCN2 is required for metastasis and coordinating crosstalk among CAFs, tumor cells, and vascular cells
4. CCN2 is a therapeutic target for cancers

Takeaway Message

Targeting the tumor stroma by blocking CCN2 action is a novel target for cancer.

FIBULIN-4 AND LATENT TRANSFORMING GROWTH FACTOR-B BINDING PROTEIN-4 CELL INTERACTIONS IN ELASTOGENESIS

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Introduction

Elastogenesis in skin represents a cell-surface-located hierarchical process that requires the recruitment of several proteins, including fibulin-4 (FBLN4) and latent transforming growth factor beta binding protein-4 (LTBP4). While it is clear that FBLN4 and LTBP4 interact with cells, the required cell receptors and the respective molecular mechanisms in elastogenesis remain unknown and are subjects of this study.

Methods

We have recombinantly produced FBLN4 and LTBP4 full length proteins, and FBLN4 deletion mutants. FBLN4 multimers and monomers were separated using gel filtration chromatography. Skin fibroblast interactions with FBLN4 and LTBP4 were assessed using cell binding assays. Heparan sulfate deficient cells and siRNA knockdowns were used to identify FBLN4 and LTBP4 cell receptors. Immunofluorescence was used to examine elastic fiber formation produced in cell culture after knocking down FBLN4 and LTBP4 cell receptors. FBLN4 and LTBP4 interactions with the ectodomains of syndecans (ED-SDCs) were analyzed using solid phase assay.

Results

Skin fibroblasts bound strongly to FBLN4 and LTBP4. FBLN4 exclusively interacted with cells as multimers. We identified two cell interaction epitopes on FBLN4 located in cbEGF2-3 and in the C-terminal domain. Cell binding to FBLN4 and to LTBP4 was entirely abolished in the presence of heparin and significantly reduced in the presence of heparan sulfate or after treating cells with heparinases. Syndecan-2 or -3 siRNA knockdown in fibroblasts abolished interaction with FBLN4, whereas only syndecan-3 knockdown abolished the interaction with LTBP4. Syndecan-2 or -3 knockdown in fibroblasts resulted in compromised elastic fiber assembly. Solid phase assays showed that FBLN4 interacts with ED-SDC2 and ED-SDC3, whereas LTBP4 only interacted with ED-SDC3. Preincubating FBLN4 with either ED-SDC2 or ED-SDC3 inhibited cell interaction, whereas preincubating LTBP4 with only ED-SDC3 inhibited cell interaction.

Conclusions

Altogether, the data identified the responsible cell-surface receptors interacting with FBLN-4 and LTBP-4, revealing a new cell-interaction role essential for proper elastogenesis in skin for these two proteins.

Learning objectives

1. To create an effective design for deletion mutants for studying protein functions including cell interaction.
2. To analyze cell interaction with extracellular elastogenic proteins (FBLN4 and LTBP4).
3. To use gene knockdown using siRNA for identifying FBLN4 and LTBP4 cell surface receptors.
4. To determine relevant changes in elastic fiber assembly by skin fibroblasts using immunofluorescence.

Takeaway Message

Skin elasticity depends on elastic fibers. Elastogenesis constitutes a hierarchical process that requires several proteins, including FBLN4 and LTBP4. FBLN4 and LTBP4 interact with skin fibroblast through syndecans. Their cell interactions are essential for elastogenesis.

SUBSTANCE P, CGRP AND AMINOGUANIDINE IMPROVE RE-EPITHELIALIZATION IN VITRO IN A GLYCATED TISSUE-ENGINEERED WOUND HEALING MODEL

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Introduction

Diabetic ulcers (DU) are major complications of diabetes. Their formation is notably caused by hyperglycemia that induces an accumulation of advanced glycation end-products (AGE) and causes skin neuropathy. To efficiently mimic diabetic ulcer characteristics in vitro, we developed a tissue-engineered skin treated with glyoxal, an AGE inducer, in which a wound was created. We studied the effect of two neuropeptides, SP and CGRP, and aminoguanidine, an anti-glycation compound, on the re-epithelialization process of this wound healing model (WHM).

Methods

The WHM was developed by culture of human fibroblast, keratinocyte and endothelial cells in a chitosan-collagen sponge. To induce glycation, the WHM was treated for two weeks with 300µM glyoxal. The wound was performed with an 8mm circular punch in the epidermal part of the model. Keratinocytes were transfected with GFP in order to monitor the reepithelization with an imaging system (IVIS).

Results

We showed that the treatment of the WHM with 300µM of glyoxal compromised the reepithelialization of the wound in contrast to the non-glycated control. This negative effect was abolished in presence of SP, CGRP and aminoguanidine supplemented in the culture medium.

Conclusions

The use of neuropeptides and anti-glycation compound can minimize the effect of AGE and offset the negative impacts of skin neuropathy on a diabetic wound. This promising approach could lead to a successful topical treatment that could lower the number of amputation due to chronic wound infection, common in diabetic patients.

Learning objectives

The participant will see:

- an example of an exploitation method of chitosan-collagen sponges
- knowledges about the effect of AGE accumulation in skin
- a deep understanding of the role of neurogenic inflammation in open wound

Takeaway Message

Tissue engineering is a promising approach for the research on skin pathologies and can be useful for further understanding of wound healing mechanisms.

FIBRILLIN-1 AND FIBRONECTIN RGD MOTIFS POST-TRANSCRIPTIONALLY REGULATE ERK1/2 SIGNALING AND FIBROBLAST PROLIFERATION VIA miR-1208

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Introduction

Mutations leading to stiff skin syndrome (SSS) localize to the Arg-Gly-Asp (RGD)-containing domain of fibrillin-1. Diffuse skin fibrosis with elevated fibroblast proliferation and TGFβ signaling represents major hallmark of SSS, which may result from compromised RGD-integrin interactions. microRNAs are short non-coding RNAs, post-transcriptionally regulating 60% of the human genes. Thus, investigation of post-transcriptional regulation of fibroblast proliferation by integrin-controlled miRNAs can shed light on SSS pathogenesis.

Methods

Fibrillin-1 and fibronectin recombinant fragments were produced by HEK293 and E. coli respectively, and purified via nickel affinity chromatography and gel filtration. microRNA were overexpressed or knocked down by transfection of mimics or inhibitors. Proliferation was assessed by immunofluorescence staining of Ki67. qPCR and Western blotting were used to examine ERK1/2 and MEK1/2 expression and phosphorylation levels.

Results

A previous study in the lab identified a subset of miRNAs regulated upon fibrillin-1 and fibronectin RGD-cell binding, with miR-1208 being the most downregulated microRNA. This study also revealed that the mRNA and protein levels of ERK1/2 and MEK1/2, the key kinases of non-canonical TGFβ signaling, were upregulated upon integrin binding. Bioinformatic analysis predicted that miR-1208 targets several sites in the 3' untranslated region of the ERK2 and MEK1 mRNAs. Reporter assays validated four miR-1208 binding sites in the ERK2, and one in the MEK1 mRNA. Western blotting demonstrated the inhibitory potential of miR-1208 on both ERK1/2 and MEK1/2. As the consequence of altered substrate levels, phosphoERK1/2 and phosphoMEK1/2 was altered accordingly upon miR-1208 treatment. miR-1208 overexpression inhibited fibroblast proliferation. Using an ERK1/2 inhibitor, we demonstrated that ERK1/2 activity was required to regulate cell proliferation by miR-1208, whereas the expression of miR-1208 was not altered by ERK1/2 activity.

Conclusions

This study identifies a novel outside-in signaling mechanism upon cell interaction with fibrillin-1 and fibronectin. This mechanism involves miR-1208, which regulates cell proliferation through post-transcriptional modulation of ERK1/2 signaling.

Learning objectives

- To investigate the involvement of microRNAs in the outside-in signaling triggered by RGD-integrin binding.
- To clarify the role of miR-1208 on ERK1/2 signaling and fibroblast proliferation.
- To study whether ERK1/2 signaling can influence miR-1208 expression.

Takeaway Message

RGD-integrin binding not only activates ERK1/2 signaling, but also post-transcriptionally regulates the availabilities of its key kinases. RGD binding downregulates miR-1208, which inhibits ERK1/2 signaling and fibroblast proliferation.

ROLE OF N-LINKED GLYCANS IN FIBULIN-5 AND LTBP-4S MEDIATED MATRIX ASSEMBLY AND FUNCTION

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Introduction

Skin requires extracellular proteins including fibulin-4 and -5 and latent TGFβ binding protein-4 (LTBP-4) long (L) and short (S) isoforms to synthesize functional elastic fibers. Mutations in these proteins cause heritable diseases such as cutis laxa, resulting in deficient elastic fibers and compromised skin elasticity. We recently revealed a dual mechanistic role of fibulin-4 in inducing stable structural extension and functional change in LTBP-4L and promoting deposition of tropoelastin onto extended LTBP-4L.

Methods

We recombinantly produced fibulin-4 and -5 and LTBP-4L and -S. Atomic force microscopy and surface plasmon resonance spectroscopy were employed to characterize conformational and binding characteristics of these proteins. Cell immunofluorescence was used to examine matrix assembly in human skin fibroblast cell cultures.

Results

We identified that fibulin-5 but not fibulin-4 can induce a similar but not identical extension and functional change to LTBP-4S, resulting in an increase in LTBP-4 assembly and tropoelastin assembly/deposition. This provides the mechanism for the previous in vivo observations suggesting the existence of two separate axes promoting elastogenesis (fibulin-4-LTBP-4L and fibulin-5-LTBP-4S). We next investigated if N-linked glycans on fibulins and LTBPs play a key role in the above-mentioned findings. N-linked glycans of fibulin-5 are essential for promoting LTBP4S binding, extension, assembly, and tropoelastin deposition. But in the LTBP-4-fibulin-4 axis, it is the N-linked glycans in LTBP-4L and not in fibulin-4, which are crucial to allow fibulin-4 mediated LTBP-4L structure, function, and assembly changes. The N-linked glycans of fibulin-4 play an inhibitory role in binding to tropoelastin and promoting tropoelastin assembly. Enzymatic or genetic removal of N-linked glycans from fibulin-4 increases its binding to tropoelastin. N-glycosylation mutants of fibulin-4, when endogenously expressed, enhances elastin assembly.

Conclusions

Our data elucidate new mechanisms which regulate the process of elastic fiber formation in skin including the fibulin-5-LTBP-4S axis and N-linked glycans.

Learning objectives

- To determine the structure function mechanism underlying the existence of two separate axes promoting elastogenesis, fibulin-4-LTBP-4L and fibulin-5-LTBP-4S.
- To identify the role of N-linked glycans in fibulin-4 mediated LTBP-4 structure, function and assembly.
- To analyze the site-specific role of N-linked glycans on fibulin-4 in tropoelastin binding and assembly.

Takeaway Message

Two axes (fibulin-4-LTBP-4L and fibulin-5-LTBP-4S) govern the formation of functional elastic fibers essential for skin. The N-linked glycans on these proteins modulate these protein interactions, their structure, function and elastic fiber formation.

NON-MELANOCYTIC CELLULAR CHANGES IN VITILIGO SKIN MICROENVIRONMENT AND THEIR CORRELATION WITH CLINICAL PHENOTYPE AND RESPONSE TO THERAPY

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Introduction

Vitiligo is an acquired depigmentation skin disease caused by immune-mediated death of melanocytes. However, the cellular changes beyond loss of melanocytes in vitiligo skin microenvironment have not been systemically investigated. The purpose of this study is to examine the changes in 64 types of non-melanocytic cells in vitiligo skin microenvironment, and test if these changes correlate with vitiligo disease phenotype and response to therapy.

Methods

With ethics approval and informed consent, we recruited 37 vitiligo patients (30 non-segmental vitiligo and 7 segmental vitiligo) and 9 individuals with healthy normal skin for this study. Each vitiligo patient had a biopsy from vitiligo lesion and the adjacent non-lesional skin at baseline, and then received twice weekly NBUVB phototherapy combined with topical tacrolimus ointment twice daily on non-phototherapy days. The patients were assessed every three months. Bulk RNA was extracted from each skin biopsy and subjected to complete RNA sequencing using Novo Gene platform, followed by cellular deconvolution analysis to yield relative abundance of melanocytes and 64 other types of cells using xCell database that contains validated gene expression signatures of these cell types.

Results

Melanocytes were missing from vitiligo lesional skin as expected. M2 macrophages, mesenchymal stem cells and 6 other cell types were also dramatically decreased. In contrast, 16 cell types including T cells, monocytes and neutrophils were increased. M1 macrophages are more abundant in lesional skin of non-segmental vitiligo than that of segmental vitiligo, and correlated with less favorable response to combined NBUVB and topical tacrolimus therapy.

Conclusions

Our results revealed previously unrecognized changes in non-melanocytic cells in vitiligo skin microenvironment that help explain heterogeneity in vitiligo clinical presentations and response to therapy.

Learning objectives

1. To find the changes in non-melanocytic cells in vitiligo skin microenvironment.
2. To learn if these changes correlate with the development and phenotype of the vitiligo disease.
3. To learn if these changes correlate with the patients' response to therapy.

Takeaway Message

How do these changes in non-melanocytic cells in vitiligo skin microenvironment affect the development, phenotype of the disease and patients' response to therapy?

COMBINATION BRAF AND MEK INHIBITION IS EFFECTIVE IN THE TREATMENT OF BRAF non-p.V600 MUTANT MELANOMAS WITH CO-OCCURRING NF1 LOSS-OF-FUNCTION OR ONCOGENIC NRAS ALTERATIONS

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Introduction

The Mitogen Activated Protein Kinase (MAPK) RAS-RAF-MEK-ERK pathway regulates cellular growth and survival. Tumour suppressors like NF1 negatively regulate this cascade by hydrolyzing active, RAS-GTP to its inactive, RAS-GDP form. Genetic studies have uncovered activating, mutations in BRAF (p.V600), N-RAS (p.G12, G13, Q61) and loss-of-function (LoF) mutations of NF1 in approximately 50%, 20% and 15% of cutaneous melanoma patients respectively. Past studies have characterized a subset of BRAF non-p.V600 mutants, that require cellular dysfunctions that increase RAS to activate the MAPK pathway. These mutants co-operate with oncogenic RAS by forming BRAF mutant/CRAF heterodimers driving MAPK activation (Yao et al., 2017). The TCGA and previous studies have reported that NF1 LoF mutations are anti-correlated with BRAF p.V600 mutations but co-occur with BRAF non-p.V600 mutations in melanoma. However, the mechanism underlying the co-operation of NF1 loss with BRAF non-p.V600 mutants compared to oncogenic NRAS is not known.

Methods

Our in vitro, signaling work was conducted in HEK293 cells with stable knockdown of NF1. These stable lines were transfected with epitope-tagged plasmids followed by subsequent immunoprecipitations and western blot analyses for corresponding MAPK protein binding and activation. Drug synergy studies were conducted using melanoma lines harbouring BRAF non-p.V600 mutations with co-occurring NF1 LoF or oncogenic NRAS mutations using cell viability as a read-out.

Results

The BRAF p.D594N mutant, found to co-occur with NF1 loss in melanoma patients, led to MAPK activation upon NF1 knockdown. Interestingly, MAPK activation of this mutant was not due to increased RAF-RAF dimer formation nor the binding to other MAPK scaffold proteins. Furthermore, using clinically approved MAPK inhibitors, we found combination BRAF and MEK inhibition effective in the treatment of BRAF non-p.V600 mutant melanomas with co-occurring oncogenic NRAS or NF1 LoF mutations.

Conclusions

We have identified a new, combination BRAF and MEK strategy to treat complex BRAF non-p.V600 mutant melanoma genotypes.

Learning objectives

1. Determine how NF1 loss co-operates with non-p.V600 BRAF mutants to modulate the MAPK pathway.
2. Dissect differences or similarities between BRAF non-p.V600 mutant signalling within an NF1 loss vs. oncogenic RAS cellular context.
3. Identify synergistic drug combinations using clinically approved MAPK inhibitors to treat complex MAPK mutant melanoma genotypes.

Takeaway Message

Using a comprehensive biochemical approach, we dissected the unique signalling of BRAF non-p.V600 mutants within NF1 loss and oncogenic RAS cellular contexts. Furthermore, we identified a combination MAPK inhibitor strategy to target melanomas harbouring complex MAPK mutant genotypes.

STAPHYLOCOCCUS AUREUS PROTEOGLYCANS CAN LIMIT TYPE 2 T CELL DIFFERENTIATION: IMPLICATIONS FOR ATOPIC DERMATITIS

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Introduction

IL-13 is a key cytokine mediating pathogenesis in atopic dermatitis patient skin. The cellular sources and accumulation sites of this cytokine remain elusive in human skin, largely due to methodological limitations. Here we introduce three validated methodologies for the detection of IL-13 in skin biopsies and skin-derived T cells of patients with atopic dermatitis.

Methods

Using confocal microscopy and flow cytometry, we show that IL-13 protein is highly sensitive to fixation with PFA, which masks key epitopes for the optimal detection by specific antibodies; this limitation requires the use of PFA-free protocols or heat-mediated protein retrieval for accurate quantification and study of this cytokine in the skin of patients. In order to distinguish between accumulation and production sites of IL-13 in human skin, we compare the localization of this cytokine at the protein level with its mRNA expression using a novel in situ hybridization method. Furthermore, we compare the production of this cytokine and its mRNA in lesional, non-lesional and ex vivo activated skin of patients with atopic dermatitis.

Results

Our results demonstrate IL-13 protein production by CD45+ cells with lymphoid morphology in the epidermis, stratum granulosum and spinosum, but more markedly in the papillary dermis. In line with this finding, the isolation and analysis of cutaneous T cells using our skin explant model and IL-13 secretion assay by flow cytometry revealed a population of memory Th2 and Tc2 lymphocytes present in lesional skin. The detection of IL-13 mRNA expression was principally found in the dermis, thus identifying distinct areas of production versus accumulation of this cytokine in the skin.

Conclusions

IL-13 mRNA expression is found to be primary dermal, while IL-13 protein can be detected in the dermis and epidermis. IL-13 protein in the epidermis co-localizes with lymphoid cells producing IL-13 mRNA as well as with keratinocytes with membrane-bound IL-13.These methods pave-the-way for more precise identification of the role of IL-13 in human skin during health and disease.

Learning objectives

Cutaneous IL-13 production remains poorly defined due to methodologic limitations. We demonstrate IL-13 mRNA expression in lymphoid cells of the papillary dermis, while IL-13 protein is found in both the epidermis and dermis in atopic dermatitis skin, reflecting epidermal lymphoid production as well as keratinocyte surface-bound IL-13.

Takeaway Message

IL-13 is a key mediator in atopic dermatitis yet cellular sources in the skin of patients remain unclear. We demonstrate IL-13 mRNA expression occurs in the papillary dermis, while protein accumulation clearly occurs in the epidermis.

Session III: Novel analytical approaches

CD109 ACTIVATES EGFR/STAT3 SIGNALING IN SQUAMOUS CELL CARCINOMA

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Introduction

Squamous cell carcinoma (SCC), is one of the most prevalent types of malignancy and its incidence is increasing globally. Despite intensive research, there has been limited success in blocking recurrence and metastasis that occurs in a sizable proportion of SCC patients. The GPI-anchored membrane protein CD109 is frequently overexpressed in squamous cell carcinoma (SCC) and this overexpression is associated with malignant transformation. Epidermal growth factor receptor (EGFR) is known to play a key role in SCC progression, and CD109 was found to potentiate EGFR signaling in lung cancer cells. We have previously shown that CD109 is a negative modulator of TGF-β signaling and responses. The aim of the current work is to understand the molecular mechanisms by which CD109 may regulate SCC progression.

Methods

In vivo, immunohistochemistry was used to study the expression levels of CD109, EGFR and STAT3 on tissue sections from CD109 knockout (ko) mice and wild type littermates (wt). In vitro, we used a SCC human cell line (A431) to generate CD109 KO. EGFR and STAT3 protein levels were assessed by western blot. The interaction of CD109 and EGFR was studied by co-immunoprecipitation.

Results

In vivo study showed that CD109 KO mice displayed decreased levels of EGFR and STAT3 signalling pathways when compared to wt as assessed by immunohistochemistry. The deletion of CD109 in human SCC (A431) cells attenuated EGFR expression and STAT3 signaling. Coimmunoprecipitation analysis demonstrated that CD109 interacts directly with EGFR. Furthermore, immunofluorescence microscopy using human SCC (A431) and CD109KO cells confirmed the co-localization of CD109 and EGFR on the cell surface.

Conclusions

Our findings showing that the loss of membrane CD109 attenuates EGFR signaling pathways both in vivo and in vitro suggests a fundamental role of CD109 in the regulation of EGFR signaling in SCC and highlight a potential clinical utility for CD109 as therapeutic target in SSC.

Learning objectives

1. the role of CD109 in squamous cell carcinomas (SSC)
2. the molecular mechanisms underlying the function of CD109 in SSC
3. In vitro and in vivo study using knockout of CD109 in mice and in cells

Takeaway Message

the CD109 plays a significant role in squamous cell carcinoma through activating the EGFR and stat3 signalling pathways

PATIENT-CENTERED DEVELOPMENT OF A MOBILE HEALTH APPLICATION FOR INTEGRATED KNOWLEDGE TRANSLATION IN ADULT ATOPIC DERMATITIS

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Introduction

Targeted immuno-therapy for adult atopic dermatitis has left an important gap in understanding both by patients and physicians alike. International guidelines involve complex regimens of skin care, topical therapies, trigger avoidance, and behavioral adaptation first line. Engaging patients is key to communicating these fundamentals; however, resources are often limited at the point of care. Here we describe the development of an implementation tool for integrated KT in order to address this gap.

Methods

A patient-engagement framework was used in order to identify the adult atopic dermatitis patient 'end-user' greatest needs. Patient partners were formally engaged and collaboratively an objective was created to survey gaps in open-source online resources. Existing educational information and online communities, as well as patient usage patterns were explored using narrative juxtaposition.

Results

A key theme was the wide breadth of unvalidated information found to dominate patient consumption. We then applied the French et al. (2012) four- step systematic framework for developing complex implementation interventions. We found that the theoretical domain of patient-knowledge was most relevant to behavioral change. Relevant barriers identified include limited expertise at point of care, absence of face-to-face KT with clinicians, fear of negative health consequences, and self-sought information overload (world wide web, social media). A validated mobile health application was determined to be the most desirable and efficient method for implementing integrated KT.We applied mixed methods to design a mobile health application with local and national patient partners. The 'Eczema.app: virtual nurse ' is a bilingual tool that features patient-driven topics, validated information, and multi-media content.

Conclusions

We found that patient knowledge was a key factor impacting first-line evidence-based management guidelines for atopic dermatitis. A novel digital tool for knowledge translation with patient partners was found to be the most desirable method for implementing iKT. A patient-engagement platform was used to co-develop a mobile health application, Eczema.app. Evidence-based education in the mobile application is tailored to the clinical context and can be personalized to the patient.

Learning objectives

Adult patients with atopic dermatitis struggle with first-line skin-directed therapy behavioural modifications and are limited by the key domain of knowledge.

Patient information-seeking behaviour is dominated by online unvalidated information. A patient-engagement platform determined that a mobile health application was most desirable for iKT, leading to the open-source Eczema.app, co-developed with patient partnership.

Takeaway Message

Patient knowledge is key for the implementation of first-line international guidelines for atopic dermatitis and must be addressed using novel tools such as a mobile health application. Eczema.app was co-developed with patient-partners for open source use.

IDENTIFYING THE CUTANEOUS MOLECULAR SIGNATURE OF CHRONIC INFLAMMATION IN ATOPIC DERMATITIS: APPROACHING PERSONALIZED MEDICINE THROUGH PROTEOMICS.

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Introduction

Atopic dermatitis (AD)became the prototypical complex condition – in which the heterogenic presentation of symptoms and difficult treatment – point towards the existence of disease endotypes. The identification of the pathogenic mechanisms ruled by different endotypes entails a significant complexity that cannot be overcome with traditional techniques. Moreover, it demands an approach providing both a holistic view and systematic results to obtain compelling information on a per-patient basis.

Methods

Here, we describe a deep proteome analysis of the lesional and non-lesional skin of an adult AD patient presenting chronically disseminated lesions in over 90% of the skin.

Results

We uncovered a total of 2546 protein sequences per each sample. The quantification of this proteomic data using the Wikipathways & Reactome databases identified significant up-regulation of the IFNɣ, IL-12 and IL-1 pathways in the lesional skin compared to non-lesional skin. In addition, the lesional skin of this patient displayed a significant activity of pathways involved in the activation of Granulocytes, whereas the TH2 pathways involving IL-4 and IL-13 were not differentially regulated. Interestingly, compared to lesional skin, the non-lesional skin showed a significant increase in RAS and MAPK proteins implicated in the regulatory pathway FLT3. Furthermore, additional results from this analysis allowed us also to identify changes in pathways and processes of the tegumentary, vascular and nervous systems occurring in the skin of this patient.

Conclusions

With this analysis, we concluded that the chronic lesional skin of this AD patient has a prominent Th1-mediated response, a significant granulocyte activity and higher activation of the IL-1 pathway compared to non-lesional skin. The results obtained with this analysis validates this approach as a robust method to identify molecular pathways implicated in the pathogenesis of AD, which can be suitable for the identification of specific disease endotypes.

Learning objectives

1. Proteome approach as a tool for identify inflammatory mechanisms and endotypes in skin diseases
2. Statistical analysis of High throughput methods
3. New methodologies to apply for personalized medicine.

Takeaway Message

Proteomic analysis is a valuable method to identify complex diseases endotypes

GEOGRAPHIC CLUSTERING OF SYSTEMIC SCLEROSIS IN CANADA AND STUDY OF POTENTIAL ENVIRONMENTAL TRIGGERS

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Introduction

Systemic sclerosis (SSc) is an autoimmune fibrosing connective tissue disease with significant morbidity and mortality, likely induced by an environmental trigger in a genetically predisposed host. In this study, we aim to investigate the presence of geographic clustering of SSc and evaluate exposure to environmental/occupational toxins leading to disease triggering.

Methods

Data was obtained from Canadian Scleroderma Research Group (CSRG) from 2003 till the present, containing 1500 patients. Forward Sortation Area (FSA) was collected for each patient and geographic maps of Canada were generated. Self-reported occupational exposures were used to compare urban and rural patients. Disease severity was estimated by autoantibody positivity, interstitial lung disease, renal involvement, and death.

Results

Of all the cases, 861 (57%) were urban and 645 (43%) were rural. Preliminary mapping results revealed areas of increased incidence not only in many urban centers, as expected, including Calgary and Edmonton, but also in rural communities such as Hudson Bay (SK), Snow Lake (MB), McAdam and Miramichi (NB). In Quebec, Salaberry-De-Valleyfield, Beaconsfield, Brome, Sherbrooke, and Alma were “hotspots”, many of which are located near paper mill factories or aluminum industry. In a case control study, we found that SSc patients living in urban areas vs. rural were less likely to be smokers (11.1% vs 16.8%, p=0.001), had an overall worse disease with 2-fold increased risk of kidney involvement (4.9% vs 2.3%; p=0.01) and presence of anti-topoisomerase antibodies (17.4 vs 12%, p=0.006). More patients in rural settings reported increased occupational exposures to paint thinners (20.3% vs 14.9%; p=0.0058), asbestos (6.4% vs 3.3%; p=0.0058), heavy metals (7.8% vs 4.8%; p=0.021) and radiation (7.9% vs 4.9%; p=0.017).

Conclusions

Preliminary analyses revealed uneven geographical distribution in Canada. Given that the CSRG database contains only ~10% of patients with SSc diagnosis in Canada, a wider population study is needed to elucidate potentially preventable and reversible environmental factors. Additionally, while in SSc occupational exposure to pollutants and smoking may be more common in patients living in rural settings, other environmental factors may account for disease characteristics in urban areas.

Learning objectives

1. Understand the geographic distribution of systemic sclerosis in Canada
2. Compare occupations and occupational exposures of patients with SSc between rural and urban settings
3. Correlate occupational exposures to disease severity and outcomes

Takeaway Message

Uneven geographical distribution of systemic sclerosis in Canada suggests certain environmental triggers play an important role in disease development and may effect disease severity and outcomes.

DEVELOPMENT OF A SKIN PIGMENTATION MODEL FOR THE STUDY OF MELANOCYTES AND PHOTOBIOLOGY

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Introduction

Skin pigmentation is a natural barrier against sun radiations. Under normal condition, melanocytes produce melanin pigments that incorporate into neighboring keratinocytes, and accumulate at a supranuclear level to withstand against ultraviolet(UV)-induced DNA damage. However, the precise mechanisms of melanin transfer and the photoprotective role of melanin still remain debated. To better understand these mechanisms, we developed an in vitro model for the study of skin pigmentation.

Methods

Using the self-assembly approach, bilayered skin constructs were produced from light- and dark-skin donor cells, with melanocytes seeded at different ratios. These reconstructed skins were characterized by histology and transmission electron microscopy(TEM). A UV radiation assay was also used to track cyclobutane pyrimidine dimers(CPDs), the most common form of photoinduced DNA damage.

Results

According to our data, the pigmented skin constructs were similar to human skin, regardless the number of seeded melanocytes. Pigmentation was observed at a macroscopic level and evidence of melanin transfer was found using TEM. After UV-radiation, the formation of CPDs in the dermis and epidermis was reduced in pigmented compared to unpigmented skin constructs.

Conclusions

Therefore, our model represents a reliable tool for the study of pigmentation processes and photobiology. Moreover, a better understanding of the pigmentation process in reconstructed skin will help develop improved skin substitutes for the treatment of burn patients. Eventually, this model will be useful for the screening of novel anticancer drugs or skin cosmetics in the future.

Learning objectives

1. Skin pigmentation is a complex process that still is not fully understood, and robust in vitro study models are lacking today.
2. Homogeneous pigmentation occurs regardless the number of seeded melanocytes in vitro.
3. CPDs does not only form in the epidermis, but also in the dermis, and melanin content is inversely correlated to their formation.

Takeaway Message

In vivo models are not a prerequisite for the study of pigmentation processes. Bilayered skin constructs produced by the self-assembly approach can mimic the human native skin and help decipher the unknown mechanisms of skin pigmentation.

EGGSHELL MEMBRANES: A COLLAGENOUS BIOMATERIAL FOR COSMETICS AND SKIN HEALTH APPLICATIONS

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Introduction

The eggshell membranes (ESM) are a fibrous meshwork of highly cross-linked proteins, which play a vital role in maintaining egg quality and protect the developing embryo in fertilized eggs. ESM is primarily composed of proteins (~90%) with a small amount of lipids (~3%) and carbohydrates (2%, mainly hyaluronic acid, HA). Proteomics analysis of ESM reveals >500 proteins including collagens, glycoproteins, avian beta-defensins, and lysozyme. The ESM exhibits various biological functions including anti-microbial, anti-inflammatory, anti-wrinkle, and anti-oxidant activities. However, utilization of this material for biomedical applications is restricted due to insolubility of ESM fiber.

Methods

Here, we present a survey of the application landscape for transformation of this unique biomaterial for skin health/cosmetic applications.

Results

The global market for collagens and HA-based biomaterials is expected to reach ~USD 9.7 billion by 2027. Industrial manufacturing of collagens is mainly based on extraction from animal tissues. However, concerns related to animal products and religious beliefs tilt consumers preference against porcine and bovine products. Further, concerns related to bovine spongiform encephalopathy (BSE) have increased the appeal of other cost-effective animal sources such as chicken. Canada produces ~9 billion eggs/year; of these, ~27% are processed at egg-breaking plants that yield low-value ESM waste as a by-product. Various methodologies including cryo-milling, enzymatic and chemical hydrolysis, and microbial fermentation must be optimized to enhance solubility of active constituents of ESM. In particular, size reduced ESM particles exhibit enhanced anti-inflammatory activity and anti-microbial inhibition of skin-associated pathogens, suggesting a great potential for modified ESM as a topical ingredient in skin care. Recently, skincare products containing ESM have become increasingly available to consumers.

Conclusions

This survey suggests that ESM is an economical source of collagens, HA, and antimicrobial proteins, which can be utilized as an eco-friendly additive to cosmetics for both antimicrobial and dermatological impact. (Supported by Egg Farmers of Canada and NSERC). Keywords: Eggshell membranes (ESM), collagens, hyaluronic acid (HA), antimicrobial and anti-inflammatory activity

Learning objectives

To survey the application landscape for transformation of ESM biomaterial for skin health/cosmetic applications.

Takeaway Message

This survey suggests that ESM is an economical source of collagens, HA, and antimicrobial proteins, which can be utilized as an eco-friendly additive to cosmetics for both antimicrobial and dermatological impact.

USE OF SEAWEEDS IN COSMETICS AND SKIN-CARE APPLICATIONS

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Introduction

The global cosmetics market is estimated to be valued approx. USD 429.8 billion by 2022, with skin-care products accounting for 39% of the industry. The cosmetics industry is highly committed to explore natural sources of ingredients from sustainable and economical raw-materials to provide innovative products to meet consumer expectations. Marine macroalgae (seaweeds) are potential raw-materials since they comprise an array of naturally occurring functional compounds that can be sourced as cosmeceutical ingredients. Seaweeds comprise approx. 25,000–30,000 species, with diversity of size, forms, pigments, and functional compounds. The global annual seaweed harvest is approx. 36 million MT, with a market size of approx. USD 6 billion and a multiplicity of various commercial applications.

Methods

Here, we present a survey of some selected seaweed-derived molecules used in skin-care applications and discuss perspectives for the future development of novel formulations.

Results

Seaweeds biosynthesize unique metabolites, which are not found in terrestrial plants. As cosmetics additives, these metabolites introduce / influence one of three major features: 1. modification of organoleptic properties; 2. stabilization and preservation; and 3. contribution of novel cosmetics function and bioactivity. Seaweed components such as sulphated polysaccharides, peptides, carotenoids, keto-type fatty acids, mycosporine-like amino acids, and flavonoids have biological effects on skin tissue such as anti-aging, anti-oxidation, moisturizing, collagen-boosting, photo-protection, melanin-inhibiting, anti-inflammatory, and antibacterial activities. In particular, sulphated polysaccharides extracted from seaweeds function as stabilizers, thickeners and emulsifiers, and are also a source of anti-microbial activities that are ideal as topical ingredients for skin-care. Yields of these active components can be improved by using robust extraction strategies, including enzyme-assisted digestion, supercritical-fluid treatment, microwave-assisted extraction and microbial fermentation. More recently, seaweed-containing skin-care products, including probiotic moisturizers (red-seaweeds) and clay masks (fucus), have been found to be preferred by consumers over synthetically sourced products.

Conclusions

This analysis suggests that seaweed-derived ingredients are cost-effective replacements for synthetic compounds in cosmetics and introduce multifunctional benefits. (Supported by institutional research funding to Dr. Hincke)

Learning objectives

a survey of some selected seaweed-derived molecules used in skin-care applications and discuss perspectives for the future development of novel formulations.

Takeaway Message

seaweed-derived ingredients are cost-effective replacements for synthetic compounds in cosmetics and introduce multifunctional benefits.

PARTICALIZED EGGSHELL MEMBRANE (PEM) FOR COSMETICS AND BIOMEDICAL APPLICATIONS

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Introduction

Eggshell membranes (ESM) provide a physical and bioactive barrier to protect the developing avian embryo via its fibrous meshwork of highly cross-linked protein components that exhibit poor solubility and reduced bioavailability. Proteomics and bioinformatics analyses reveal that the collagen-rich ESM is composed of >500 proteins with attributes of antimicrobial, anti-adhesive, immune-modulating, anti-cancer, anti-hypertensive, and antioxidant functionalities.

Methods

The goal of this study was to produce novel ESM particles (PEM) with enhanced anti-bacterial, anti-inflammatory, and anti-oxidation bioactivities for a focus on positive skin health. A novel top-down method was developed to produce particalized ESM (PEM) from table eggs, in a size range approaching nanoscale, by a combination of cryo-milling / cryo-grinding, emulsifying, and freeze-drying processes.

Results

A novel top-down method was developed to produce particalized ESM (PEM) from table eggs, in a size range approaching nanoscale, by a combination of cryo-milling / cryo-grinding, emulsifying, and freeze-drying processes. PEM exhibited a size-dependent bactericidal activity against Gram-positive Staphylococcus aureus (S. aureus; 4.5 ± 0.3 log10 inhibition), and bacteriostatic effect against Gram-negative Pseudomonas aeruginosa (P. aeruginosa; 2.1 ± 0.2 log10 inhibition) and Escherichia coli (E.coli; 1.5 ± 0.4 log10 inhibition) species. A dose-dependent antimicrobial activity for PEM (<15 μm) was observed against S. aureus and E.coli. Also, a significant dose-dependent anti-inflammatory activity was observed for LPS-induced NO production by RAW 264.7 macrophages, for PEM particles <15 μm (24.7 ± 7.0% inhibition) and <53 μm (45.2 ± 13.4% inhibition). Our preliminarily study demonstrated that processed ESM showed higher anti-oxidant capacity than egg white.

Conclusions

Taken together, our green chemistry approach for the production of PEM has great potential for the development of a novel topical ingredient for cosmetics/ skin care applications, with antioxidant, antimicrobial and anti-inflammatory features. (Supported by Egg Farmers of Canada and NSERC).

Learning objectives

To produce novel ESM particles (PEM) with enhanced anti-bacterial, anti-inflammatory, and anti-oxidation bioactivities for a focus on positive skin health. A novel top-down method was developed to produce particalized ESM (PEM) from table eggs, in a size range approaching nanoscale, by a combination of cryo-milling / cryo-grinding, emulsifying, and freeze-drying processes.

Takeaway Message

Our green chemistry approach for the production of PEM has great potential for the development of a novel topical ingredient for cosmetics/ skin care applications, with antioxidant, antimicrobial and anti-inflammatory features.

A NEW 3D VASCULARIZED SKIN MODEL CONTAINING IMMUNE CELLS RECONSTRUCTED BY TISSUE ENGINEERING

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Introduction

In vitro three-dimensional (3D) human skin models is a great tool for a better understanding of biological and pathological processes. It has already proven its worth for tests in pharmaceutical drug toxicity and efficiency. However, the majority of current in vitro models lack an immune system, vasculature, and other characteristics of native skin, limiting our capacity to understand all cellular interactions.

Methods

We developed a cell extraction technique that allows to isolate all types of cells from the same donor (fibroblasts, keratinocytes, immune and endothelial cells). From that isolation and for the first time, a 3D skin model containing immune and endothelial cells has been developed. Skin biopsies were enzymatically treated to independently isolate epidermal and dermal cells. First, fibroblasts were cultured for three weeks with ascorbic acid stimulation to produce extracellular matrix in order to obtain a cell sheet. The dermal fraction containing immune cells and endothelial cells of autologous origin, was then seeded onto one cell sheet while the epidermal fraction was seeded on another cell sheet. The two sheets were then superimposed and raised at the air-liquid interface to stimulate epidermis differentiation.

Results

Two weeks later, histological studies of our model showed a stratified epidermis on a dermis composed of cells embedded inside a matrix. Immunofluorescence staining highlighted viable autologous CD45+ immune cells, CD3+ lymphocytes, tryptase+ mastocytes, CD14+ CD68+ monocytes/ macrophages and an auto assembling capillary network (CD31+) in the dermis.

Conclusions

Creation of this first autologous 3D vascularized model containing immune cells will increase our knowledges into skin homeostasis as well as in diseases.

Learning objectives

Being able to isolate immune cells from the dermis, being able to maintain immune cells in 3D skin model. Create a 3D skin model for the understanding of all cutaneous cell's interactions in skin. Having a new tool for the understanding of immune cells regulation in skin homeostasis and pathology.

Takeaway Message

For the first time we are now able to isolate all cells types of a same donor and to produce an autologous 3D skin model with capillary network and immune cells.

Session IV: Clinical and therapeutic considerations

EFFECTS OF SODIUM BUTYRATE ON VASCULARISATION AND MATRIX REMODELLING IN A MODEL OF GRANULATION TISSUE IN MICE - INVOLVEMENT OF THE RECEPTOR GPR43

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Introduction

Butyrate is a short-chain fatty acid derived from microbiota and is involved in a range of cell processes in a concentration-dependent manner, including features associated with granulation tissue formation such as angiogenesis. However, the mechanisms associated with these effects are not yet fully known. Here, we investigated the effects of a low dose of sodium butyrate (NaBu) on the fibrovascular tissue induced by synthetic implants in mice lacking or not the GPR43 gene.

Methods

Polyether-polyurethane sponges were subcutaneously implanted in C57BL/6 GPR43-KO (knock-out) or GPR43-WT (wild-type) male mice that received 40 µl of NaBu (1.0 µg/sponge) or PBS (Vehicle) intra-implant immediately after the surgery, and daily until the 3rd day post-implantation. At day 7, the animals were anesthetized for blood flow evaluation (Laser Doppler Perfusion Imaging) in the sponges, and then killed by anesthetic overdose. The implants were collected for hemoglobin content - Drabkin method, VEGF and TGF-β1 levels - ELISA, α-SMA immunohistochemistry, and histological analysis (Hematoxylin and eosin, PAS/Alcian Blue, and Picrossirius red/polarized-light microscopy). L929 murine fibroblasts were treated with NaBu in vitro and migration was evaluated by scratch wound assay.

Results

Our results show that NaBu enhances new blood vessel formation, VEGF levels, and blood flow in a GPR43-dependent manner in the implants. Moreover, NaBu was able to modulate tissue remodeling aspects of the granulation tissue such as proteoglycans production, collagen deposition, TGF-b1 levels, and α-SMA expression in a GPR43-dependent manner. In vitro, NaBu stimulated fibroblasts migration and production of TGF-b1, suggesting a direct effect of NaBu on fibroblast activation.

Conclusions

Overall, our data suggests the contribution of the NaBu/GPR43 axis in promoting angiogenesis, fibroblast activation, and extracellular matrix remodeling during granulation tissue formation in mice.

Learning objectives

- Evaluate the effects of the sodium butyrate (NaBu), the mainly form of the short-chain fatty (SCFA) acid Butyrate, on angiogenesis and tissue remodelling in vivo;
- Evaluate the involvement of the receptor GPR43 on sodium butyrate (NaBu) effects;
- Evaluate the effects of NaBu on fibrobasts in vitro.

Takeaway Message

NaBu stimulates angiogenesis and tissue remodelling in a GPR43 manner.

ASSESSMENT OF T-CELL REPERTOIRE IN NON-HODGKIN'S T-CELL LYMPHOMAS

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Introduction

T-cell lymphomas contributed towards 10-15% of all non-Hodgkin's lymphomas. The diagnosis includes bone marrow testing, identification of histological markers and analysis of T-cell repertoire. Most of the T-cell lymphomas are considered to be developed from mature T-cells and therefore, identification of a dominant T-cell receptor (TCR) clonotype is a diagnostic confirmation of TCLs. Yet, in certain samples TCR analysis has identified samples with absence of dominant TCR clonotypes questioning the validity of TCLs developing from mature monoclonal T-cells. The aim of this study is to assess the TCR profile in different types of TCLs to validate the theory of mature monoclonal T-cell as the origin of TCLs.

Methods

We meta-analyzed 400 samples of whole genome, exome and transcriptome sequencing data to identify the TCR repertoire profile and tumor cell fraction (TCF) from previously published 14 studies. The current method of amplification and sequencing of TCR cannot distinguish malignant from reactive TCR clonotypes. Most studies classify the dominant clonotype as malignant or use arbitrary thresholds to classify the malignant from reactive TCRs. We instead propose the use of TCF to compare and identify the malignant clonotypes.

Results

Analysis of the genome and transcriptome data identified multiple malignant TCRβ clonotypes across all subtypes of TCLs.

Conclusions

In conclusion, most of the TCLs samples present a oligoclonal or a polyclonal malignant TCR profile thus challenging the theory of mature monoclonal T-cell as the origin of the TCLs.

Learning objectives

Lack of better methods to classify malignant and reactive TCR clonotypes has been a limitation for T-cell lymphomas. Use of Tumor cell fraction (TCF) to compare and identify the malignant clonotypes can eliminate the need for arbitrary thresholds.

Takeaway Message

T-cell lymphomas (TCLs) presents multiple malignant T-cell clonotypes and thus an immature precursor T-cell is likely to be the origin cell in TCLs.

PRELIMINARY RESULTS FROM THE HIDRADENITIS SUPPURATIVA PATIENT EXPERIENCE (Hspe) SURVEY

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Introduction

Hidradenitis suppurativa (HS) is a devastating skin condition, characterized by recurrent nodules and abscesses in skin folds, affecting up to 4% of the population. A comprehensive examination of the patient experience was undertaken to track future progress in improving care for individuals with HS.

Methods

In January 2020, we conducted an online survey of individuals with HS. To disseminate the survey, we engaged HS-related patient advocacy groups, physician groups, and social media groups. Survey questions included: time to diagnosis, number of healthcare encounters, disease-related costs, and impact on vocational and personal life.

Results

To date, 163 eligible respondents have completed the survey, of which 34 were Canadian. The majority of Canadian respondents (92.6%) were female and the mean age of diagnosis was 28.7 years (SD = 12.3, range 13-71). The average amount of time from symptom onset to diagnosis for Canadian respondents was 8.6 years (SD = 12.2, range = 2 months - 50 years), with 57.6% having had at least 7 physician visits prior to diagnosis. The majority of Canadian respondents (61.3%) were dissatisfied or extremely dissatisfied with their healthcare experience prior to diagnosis, but this number decreased to 41.9% once a diagnosis was made.

Analysis of all 163 respondents showed that 84% had at least one misdiagnosis, of which boils and ingrown hairs were most common. 43.2% of all respondents felt that their pain was mostly or very uncontrolled. Furthermore, 90% of all respondents felt that HS affected their social life, and 87.7% felt that HS affected their intimate/sexual relationships. Respondents struggled the most with managing symptoms, lack of awareness among physicians, as well as managing depression and anxiety.

Conclusions

Our preliminary data highlight the psychosocial impacts of HS and the importance of timely diagnosis and greater awareness of this condition among primary healthcare providers.

Learning objectives

1. Canadian HS patients experience a diagnostic delay averaging 8.6 years.
2. The majority of HS patients were dissatisfied with their healthcare experience prior to diagnosis, but this number decreased once a diagnosis was established.
3. Many HS patients had at least one misdiagnosis.
4. Majority of HS patients felt that symptoms impacted their social life and intimate relationships.

Takeaway Message

Our preliminary data highlight the psychosocial impacts of HS and the importance of timely diagnosis and greater awareness of this condition among primary healthcare providers.

ANTIHYPERTENSIVE MEDICATION USE AND RISK OF SKIN CANCER

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Introduction

Antihypertensive medications may be associated with an increased risk of skin cancer, but studies have had heterogeneous results and are limited by small sample sizes and methodological biases. To better characterize the association, we conducted population-based cohort studies using administrative data in Ontario, Canada.

Methods

We included users of antihypertensive medications aged ≥66 years with a first prescription (index date) from 1997 to 2015. For each user we identified two age- and sex-matched controls who filled a non-antihypertensive prescription within 30 days of the index date. During follow-up, controls became users if they subsequently filled an antihypertensive prescription. We categorized each antihypertensive class by cumulative annual standard dosage (ASD = WHO Daily Defined Dose*365 days). The outcomes were time to first keratinocyte carcinoma (KC), first advanced KC (fatal KC or KC >2cm diameter, requiring complex surgical repair or lymphadenectomy) and first melanoma. We used cause-specific hazards regression models to calculate adjusted hazard ratios (aHR) and 95% confidence intervals (CI) for the risk of each outcome associated with each antihypertensive class. Models included important covariates and a binary ever-use variable for each class to adjust for selection bias.

Results

We included 378,472 and 451,031 exposed patients in the KC and melanoma cohorts, respectively, matched to and 756,944 and 902,062 controls. We found a dose-response relationship for thiazides and risk for KC (aHR for >7.5 ASD vs. 0-0.5 ASD: 1.44, 95% CI 1.36 to 1.52) and advanced KC (aHR for >7.5 ASD: 1.57, 95% CI 1.37 to 1.79) but no significant association with melanoma. For other antihypertensive classes, associations were either weak or null.

Conclusions

Phototoxicity has been proposed as the mechanism for the association between thiazides and KC; it may be beneficial to promote sun protection for patients taking thiazides and to limit thiazide exposure in immunosuppressed and other high risk patients.

Learning objectives

1. Investigate the potential association between antihypertensive medication use and keratinocyte carcinoma.
2. Investigate the potential association between antihypertensive medication use and melanoma.
3. Develop clinical implications of the association between thiazide diuretics and keratinocyte carcinoma.

Takeaway Message

In this population-based study, thiazide diuretics were associated with risk for subsequent keratinocyte carcinoma. These findings support a recent Health Canada warning and should encourage sun protection among thiazide users.

SEX DIFFERENCES IN PATTERNS OF SYSTEMIC AGENTS' USE AMONG INDIVIDUALS WITH PSORIASIS

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Introduction

Psoriasis severity differs between men and women. However, it is not clear if psoriasis management also differs by sex. Severe psoriasis is treated with conventional systemic agents (CSA) and/or biologic agents when CSA are ineffective or inappropriate. We assessed sex differences in the utilization patterns of CSA and switches to biologic agents among individuals with psoriasis.

Methods

We conducted a retrospective cohort study using Quebec health administrative databases (1997 - 2015). Individuals ages ≥ 20 years were included if they received a first psoriasis diagnosis and at least one CSA. Individuals were followed for two years. Treatment switch (to another CSA or a biologic agent) and discontinuation were examined. Multinomial logistic regression models were conducted to identify predictors of treatment switch and discontinuation. Analyses were stratified by sex.

Results

We included 1,272 individuals with psoriasis (55% females). Most individuals were initiated on methotrexate (MTX, 56.4%), followed by acitretin (36.8%). During the follow-up, 364 (29%) were persistent, 688 (54%) discontinued and 220 (17%) switched (75% to another CSA and 25% to a biologic agent). Most patients switching to a biologic agent received adalimumab (41%) and infliximab (27%). Compared to females, males tended to switch more to a biologic agent (44/1000 person-years vs 27/1000 person-years). When compared to persistent patients, males and females who switched were more likely to be younger and initiated on acitretin and sulfasalazine (vs MTX). Males with psoriatic arthritis and females with rheumatoid arthritis were less likely to discontinue their initial CSA. We are currently conducting a clustering analysis (unsupervised machine learning technique) to portray the dynamic changes in therapy over time by creating groups with similar patterns.

Conclusions

MTX was the CSA treatment of choice for psoriasis. Persistence on CSA was poorer among males, younger psoriasis patients and higher among those with other inflammatory diseases.

Learning objectives

- Describe utilization patterns of CSA and switches to biologic agents among individuals with psoriasis.
- Examine if there are sex differences in (1) prescribing systemic agents to individuals with psoriasis, and (2) persistence to these agents.
- Determine the predictors of treatment switch and discontinuation

Takeaway Message

The pattern of systemic agents' use in psoriasis is dynamic in both sexes. Psoriasis patients with other inflammatory diseases and those of younger age should be better monitored by health care professionals because they are less likely to be persistent.

THE RELATIONSHIP BETWEEN MEIOMITOSIS, THE meiCT GENE HORMAD1 AND GENOMIC INSTABILITY IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS

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Research Institute of McGill University

Introduction

Genomic instability has been called a facilitating characteristic for the hallmarks of cancer, however the dynamic mechanisms that drive genomic instability remain elusive. Research demonstrates that many cancers with high levels of genomic instability also ectopically express meiosis genes in a process called meiomitosis. Cancer meiomitosis is the orchestrated activation of both mitotic and meiotic machineries in neoplastic cells that confers a selective advantage. MeiCT (meiosis-specific cancer/testis) genes that specialize in the reductional division of germ cells in meiosis I are ectopically expressed in several cancers, particularly in squamous cell carcinomas. We focus on the meiCT gene, HORMAD1, a meiosis specific protein that functions to ensure that a sufficient number of DSBs are formed for proper meiotic progression and to maintain genome integrity throughout the process of homologous recombination. Studies show that HORMAD1 is significantly upregulated in several cancers and is regarded as a potentially important oncogene that plays a role in sustaining increased genomic instability.

Methods

With the use of shRNA mediated knockdown of HORMAD1, we evaluated the effects of HORMAD1 on genomic instability and survival in head and neck squamous cell carcinoma (HNSCC) following treatment with etoposide, a topoisomerase that facilitates DNA strand breaks.

Results

Our results demonstrate that HORMAD1 expression in HNSCC cancer cell lines sustains a functional level of genomic instability by decreasing the number of double strand breaks, chromatin bridges, and micronuclei, leading to increased cell survival in response to DNA damage elicited by etoposide treatment.

Conclusions

We conclude that HORMAD1 modulates a functional and balanced level of genomic instability, likely by eliciting an DNA damage response that allows HNSCC to survive and proliferate following DNA damage.

Learning objectives

1. To evaluate the influence of HORMAD1 expression on markers of genomic instability in HNSCC.
2. To identify changes in genomic instability following treatment with etoposide.
3. To analyze the relationship between changes in genomic instability and HORMAD1 expression following etoposide treatment.

Takeaway Message

HORMAD1 helps cancer cells mediate DNA damage and avoid detrimental levels of genomic instability. Therefore, HORMAD1 is be a potential novel target to treat cancers with high levels of genomic instability that often acquire chemoresistance.

PRIMARY CUTANEOUS CD4+ SMALL/MEDIUM-SIZED PLEOMORPHIC T-CELL LYMPHOPROLIFERATIVE DISORDER: THE ALBERTA PERSPECTIVE AND REVIEW OF THE LITERATURE

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Introduction

Primary cutaneous CD4+ small/medium pleomorphic T-cell lymphoproliferative disorder (PCSM-LPD) was reclassified as a provisional entity by the WHO in 2016 due to its uncertain malignant potential. As it becomes better known, the rate of diagnosis appears to be increasing. In 2011, 237 cases of CD4+ PCSM-LPD were reported in literature worldwide, and in 2017 this number increased to 529. While its estimated prevalence is 2% of all cutaneous T cell lymphomas, its prognosis, clinical characteristics, and histopathology are still in dispute and an emerging theory postulates an etiology based on a reactive phenomenon. Currently, no criteria exist to discriminate between indolent and aggressive cases of CD4+ PCSM-LPD, and there is no consensus on the appropriate treatment or surveillance protocol.

Methods

A retrospective review of 26 patients in Calgary and Edmonton diagnosed with CD4+ PCSM-LPD from 2000- 2019 was performed. We included only patients who fulfilled clinical and histopathological criteria of CD4+ PCSM-LPD as defined by the WHO-EORTC classification. Clinical data including age at diagnosis, gender, clinical features, treatment, follow-up, and outcome were assessed. Patients with alternate diagnoses were excluded.

Results

12 patients with CD4+ PCSM-LPD were identified in Calgary and 14 in Edmonton. CD4+ PCSM-LPD presented predominantly with solitary lesions localized to the head/neck and demonstrating monoclonality of both beta and gamma T-cell receptors. Relapses occurred in 2 patients, with surgical excision being the most effective technique for preventing relapse.

Conclusions

CD4+ PCSM-LPD represents a poorly described and heterogeneous condition that may represent a diagnostic and therapeutic challenge for practitioners. Herein, we characterize 26 patients observed in Calgary and Edmonton academic centers, with a focus on describing patient characteristics, therapeutic outcomes, and histopathological features. These cases help to further characterize CD4+ PCSM-LPD and add to the growing body of literature on this provisional entity.

Learning objectives

1. Describe the existing research currently surrounding CD4+ PCSM-LPD.
2. Outline Calgary and Edmonton's experience with CD4+ PCSM-LPD patient and disease characteristics.
3. Describe the histopathological features observed in Alberta cases of CD4+ PCSM-LPD.
4. Provide the clinician with a more thorough understanding into recognizing CD4+ PCSM-LPD and appropriate next steps.

Takeaway Message

Primary cutaneous CD4+ small/medium pleomorphic T-cell lymphoproliferative disorder has only recently been recognized as a unique entity and represents a diagnostic/therapeutic challenge. Herein, we characterize 26 Alberta patients and describe cohort characteristics, therapeutic outcomes, and histopathological features.

Poster Session

THREE-DIMENSIONAL BODY IMAGING OF SKIN DISEASES

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Introduction

Skin disease is one of the most common human illnesses and affects people from all demographic backgrounds. A crucial part of skin disease management is tracking its progression in a consistent and standardized manner. However, the landscape of the human skin takes an extremely complex form and can make it difficult for dermatologists to achieve this quantitatively.

Methods

Patients were recruited and consented from the University Dermatology Centre in the Kaye Edmonton Clinic. Short video recordings were taken while the patient rotated in front of a fixed camera lens. In order to achieve the most accurate and complete mapping of the body, 3 different arm positions were held. Videos were then pre-processed in the Pixelmator application to extract the image frames, and processed using machine learning algorithms (i.e. deep learning and convolutional neural networks) to create 2D renderings of our patients' skin.

Results

Our 2D model deconvoluted the patient's skin and allowed us to view it as a flat map. Body silhouettes and segmentation were completed in the front and side views. Image masks were also created from the input media. As our study progresses, the mapped results will be used to measure and quantify psoriasis lesions on each patient's body.

Conclusions

The 2D model deconvolutes the patient's skin and allows us to view it flatly. We are currently in the process of creating a 3D model of patients' skin using the renderings of the 2D map.

Learning objectives

1. Recognize the qualities of human skin that make it difficult to track dermatological disease progression in a standardized manner.
2. Understand the use of novel machine learning techniques to map the surface area of skin lesions on a model of the human skin.
3. Apply the rendered skin map in the context of monitoring psoriasis plaque progression.

Takeaway Message

Machine learning is a valuable tool in tracking the prognosis and treatment of skin conditions.

IN VIVO QUANTIFICATION OF NITRIC OXIDE (NO) RELEASE FROM INTACT HUMAN SKIN FOLLOWING EXPOSURE TO PHOTO BIOMODULATION WAVELENGTHS IN THE VISIBLE AND NEAR-INFRARED SPECTRUM

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Introduction

Human skin contains photolabile nitric oxide (NO) derivatives which decompose after UVR irradiation and release vasoactive NO. However, aside from blue light, barely nothing has been reported about the effects of red and NIR wavelengths upon this NO bioactivity. We decided to investigate if photobiomodulation, using visible to NIR light, would increase the release of NO from their skin.

Methods

A custom-built airtight sleeve, which envelopes the subject's forearm, was used to measure the NO emanating from the skin under photobiomodulation conditions (660nm and 850nm at 50 mW/cm2) and quantified by chemiluminescence detection (Sievers 280i, Zysense, Weddington, NC, USA)

Results

Distinct differences in measured NO levels were observed between the non-irradiated condition and PBM conditions. As anticipated, blue light released the largest amount of NO, since it is very close to UVA. Surprisingly, near-infrared (NIR) at 850nm released twice as much NO as visible red light at 660 nm.

Conclusions

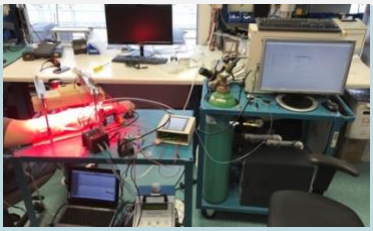
This preliminary pilot study has demonstrated real-time gaseous NO release from skin using harmless red and NIR wavelengths. Moreover, we compared the ionizing radiation to the non-ionizing radiation impacts upon NO bioactivity in the skin. NIR showed a remarkable ability to release high amount of NO avoiding skin photo-damage.

Learning objectives

1. To assess if PBM conditions are able to release sufficient amount of NO in the skin.
2. To assess if non-ionizing PBM conditions can be compared to Ionizing conditions in terms of NO bioactivity in the skin.
3. To establish a basal set-point of NO released from the skin following different irradiation conditions.

Takeaway Message

Blue light releases the highest amount of NO from the skin. However, NIR could be considered as a possible alternative to this ionizing blue light. 850 nm is able to release comparable amount of NO avoiding skin photo-damage.



STAPHYLOCOCCUS AUREUS PROTEOGLYCANS CAN LIMIT TYPE 2 T CELL DIFFERENTIATION: IMPLICATIONS FOR ATOPIC DERMATITIS

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Introduction

We previously reported that Staphylococcus aureus isolates exhibit heterogeneity in their immunomodulatory capacity. Here we investigated the capacity for cell wall components of S. aureus to regulate human Th2-type immune responses associated with atopic dermatitis.

Methods

Using a novel in vitro model, human type 2 T cells were generated from peripheral blood mononuclear cells using molecular factors relevant to atopic dermatitis skin inflammation. S. aureus strains, their isolated cell walls, and associated pathogen-associated molecular patterns were then tested for modulation of memory Tc2 and Th2 cells differentiation, applying multiparametric flow cytometry, Imagestream analysis and secretion assays. Inhibitory capacity was validated in vivo, with a mouse model of allergic skin inflammation, as well in disease, with circulating immune cells isolated from patients with atopic dermatitis.

Results

Heat-killed S. aureus isolates inhibited the expansion of IL-13-secreting memory Tc2 and Th2 cells. This inhibitory effect was reproduced using either purified cell wall of a S.aureus isolate, or wall-associated molecular patterns. The NOD ligand PGN-SAndi restricted the generation of Th2 cells and reduced allergic sensitization in an atopic dermatitis murine model. Memory type 2 T cell inhibition was dependent on reduced IL-2 production and the activation of STAT3 signaling. This inhibitory effect could be reproduced in cells isolated from atopic dermatitis patients.

Conclusions

We demonstrate here that TLR2 and NOD ligands in the cell wall of S. aureus trigger to mechanisms inhibiting type 2 T cell differentiation in vitro thereby limiting skin inflammatory responses in vivo.

Learning objectives

- S. aureus plays an important role in atopic dermatitis
- Host-microbial interactions in the skin include anti-inflammatory mechanisms
- Exposure to S. aureus cell wall components can limit Th2 responses
- A fragment of the most abundant S. aureus protein, peptidoglycan, signals through NOD, an innate pattern recognition receptor
- NOD signaling induces STAT3 signaling, thus inhibiting Th2 responses.

Takeaway Message

S. aureus cell wall components inhibit human Th2 and Tc2 cell differentiation and IL-13 production, both in normal subjects and in atopic dermatitis patients.

GEOGRAPHY, THE BUILT-IN ENVIRONMENT, AND THEIR RELATIONSHIP WITH MELANOMA INCIDENCE DISTRIBUTION ACROSS CANADA

Berman-Rosa, M., Al-Ghazawi, F., Savin, E., Litvinov, I., et al., (2020).
McGill University Faculty of Medicine

Introduction

Despite its preventability, the incidence of cutaneous malignant melanoma (CMM) continues to increase worldwide. The primary and most avoidable risk factor for skin cancer is exposure to solar ultraviolet radiation (UVR). Recent population-based analyses of cases of CMM in Canada throughout the years 1992-2010 have revealed a steady increase in the incidence of CMM across the Canadian provinces and territories. Identifying features in the environment that may augment the risk of UV exposure is important for targeted public health strategies seeking to reduce melanoma risk.

Methods

Remote sensing technology is used widely for investigating vegetation distribution, local climate zones, ultraviolet solar radiation, and precipitation across Canada. Modelled UVR, shade, and precipitation data for the period of 1992 to 2010 will be spatially linked with Geographic Information Systems by forward sortation areas (FSAs) across Canada and a risk-map distribution will be created for each environmental risk factor. Correlation analysis of melanoma incidence rates over time and environmental risk factors will follow.

Results

We hypothesize that the FSA's with greater burden of risk (less shade availability, less precipitation, greater UVR) will strongly correlate with those FSA's identified to have had the greatest increase in incidence of melanoma compared to the Canadian average between 1992-2010.

Conclusions

Identification of shade availability and the magnitude of environmental exposure to melanoma risk factors in FSA's across Canada will aid in the identification of geographical 'risk-zones' where more targeted public health interventions for reducing melanoma incidence could be applied.

Learning objectives

1. Identifying distribution of environmental risk factors for melanoma across Canada
2. Exploring how risk factors may be associated with melanoma incidence rates across Canada
3. Identifying forward sortation areas for targeted public health interventions.

Takeaway Message

Despite its preventability, the incidence of cutaneous malignant melanoma continues to rise across Canada. Supportive environments offer protection from factors that can threaten good health. We hope to identify geographical risk-zones for melanoma to aid direct future interventions.

A VARIANT OF MOHS MICROGRAPHIC SURGERY: THE MUFFIN TECHNIQUE

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Introduction

Moh's micrographic surgery (MMS) is the gold standard treatment for non-melanoma skin cancers (NMSC) such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Despite the advantages of MMS, the technique also has limitations. The procedure can be time-consuming and, due to the bulky nature of many cutaneous tumors, appropriately sectioning and flattening the specimen for cryosection without omitting tissue is difficult. A novel method has recently been proposed as an alternative to the traditional MMS approach. Due to the procedure's similarity to the removal of the paper lining encasing a muffin, it has been aptly named the "Muffin Technique". Herein, we describe the "Muffin Technique" and outline the procedure's safety and effectiveness in a real-world institution.

Methods

We conducted a retrospective chart review of all patients with BCC or SCC who underwent MMS with the Muffin Technique at the University of Alberta Dermatology Centre from June 2016 until September 2019. Details on demographics, disease burden, and procedure effectiveness and complications were analyzed and compared to existing MMS data to compare this variant technique to the traditional approach.

Results

A total of 69 patients were included with 64 BCCs and 5 SCCs who underwent Muffin Mohs. There were no major procedural complications, and 68.1% of the surgeries had clear margins after the first incisions, 100% after second round re-excisions. Muffin proved effective in the excision of high-risk recurrent and larger tumors. Surgical wounds were smaller than with traditional approaches. All patients remain disease-free upon most recent follow-up.

Conclusions

The Muffin Technique represents a variation on the traditional Mohs approach and enables paraffin fixation that may be stored for pathologic evaluation for longer, allows for larger specimens to be evaluated on a single slide, and possesses advantages for smaller incisions and wound healing. We hope to introduce the field of dermatology to this alternative surgical approach to ultimately improve patient care.

Learning objectives

1. Discuss traditional Mohs Micrographic Surgery and its indications
2. Outline current limitations of traditional Mohs methodologies
3. Introduce the Muffin variant of Mohs Micrographic Surgery
4. Describe the advantages of the Muffin technique over traditional Mohs
5. Discuss the efficacy and safety of Muffin Mohs in a real Canadian cohort

Takeaway Message

Despite boasting the lowest rates of non-melanoma skin cancer recurrence, Mohs is limited by the incision techniques and cryostat preparation of its slides. A novel variant termed the Muffin method addresses these limitations while retaining excellent efficacy and safety.

SEX DIFFERENCES IN THE RISK OF DIABETES MELLITUS AMONG INDIVIDUALS WITH PSORIASIS: A RETROSPECTIVE COHORT STUDY IN QUEBEC, CANADA

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Introduction

Psoriasis severity is associated with diabetes mellitus (DM) and severe psoriasis is more common in male than female individuals. Sex differences in the risk of DM among individuals with psoriasis has not been examined.

Methods

A retrospective cohort study was conducted using Quebec health administrative databases (1997-2015). Psoriasis treatments served as surrogates for disease severity [untreated, mild (topical agents), moderate (phototherapy), severe (systemic agents)]. Time-dependent multivariate Cox regression models (with 12-month lag) were used. Sensitivity analyses were conducted using other definitions for DM, different lag periods, and censoring individuals receiving methotrexate and cyclosporine that have been associated with DM.

Results

This study included 11,236 individuals with psoriasis (52.4% female individuals). Unadjusted DM incidence was higher in male (versus female) individuals (20/1000 person-years vs 17/1000 person-years). DM risk increased with psoriasis severity in both sexes (severe vs untreated individuals: hazard ratio; 95% confidence interval: males 2.16, 1.44-3.25; and females 2.27, 1.42-3.63). The effect of age on DM differed between sexes and psoriatic arthritis was associated with DM only in male individuals (1.57, 1.10-2.24).

Conclusions

Screening for DM should be considered for both sexes with high psoriasis severity and for male invdividuals with psoriatic arthritis.

Learning objectives

1. Determine the incidence of diabetes mellitus among individuals with psoriasis living in Quebec
2. Assess differences in the risk of DM by psoriasis severity in both sexes.
3. Determine the predictors of diabetes mellitus in male and female individuals with psoriasis.

Takeaway Message

Psoriasis is an established risk factor for diabetes mellitus. Our findings demonstrate that the risk of diabetes mellitus increases with psoriasis severity in both sexes, and psoriatic arthritis predicts diabetes mellitus only in male individuals with psoriasis

MIXED PROGESTOGEN HYPERSENSITIVITY IN A PATIENT USING A COMBINED CONTRACEPTIVE SKIN PATCH: A CASE REPORT

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Émilie Proulx, ***Centre hospitalier régional de Lanaudière, Saint-Charles-Borromée (Joliette), QC***

Introduction

Progestogen hypersensitivity (PH) is a rare disorder usually affecting women of reproductive age. It can appear with a spectrum of cutaneous/systemic hypersensitivity manifestations, correlating with peaks in serum progesterone levels. The clinical presentation is variable, with IgE- and non-IgE-mediated hypersensitivity symptoms having been reported. No estimates of incidence or prevalence are currently published.

Methods

A 21-year-old Caucasian woman with a past medical history of urticaria (dermographism), allergic rhinitis and allergic asthma was referred to our clinic for recurrent anaphylaxis three weeks after starting a combined contraceptive skin patch (norelgestromin/Ethinyl estradiol, N/E).

Results

Two days after the first application, sneezing, coughing and nasal congestion occurred. Two days after the second patch, an acute asthma exacerbation with facial angioedema emerged, initially improved by antihistamines, but culminated in full-blown anaphylaxis after alcohol consumption. She was treated with IM epinephrine, antihistamines and a course of prednisone. Urticaria recurred at the application site after reusing the patch four days later. Allergy skin prick testing was positive for both N/E and levonorgestrel, previously used as contraception, but negative for various other oral contraceptives. In-patient oral challenge to norgestimate/Ethinyl estradiol was well tolerated. D816V c-kit mutation on peripheral blood was negative. The patient was sent home on norgestimate/Ethinyl estradiol for contraception and rupatadine for dermographism and allergic rhinitis. Another episode of anaphylaxis occurred 5 days later, after exposure to cat dandruff. Exogenous progestogen's use was stopped thereafter, without any recurrence of mast cell activation symptoms.

Conclusions

Diagnosing PH is challenging due to its various clinical presentations and a lack of standardized skin testing. This case highlights:(1)the co-existence of IgE- and non-IgE-mediated mast cell activation in a patient sensitized to estrane progestins and clinically reactive to 13-ethylgonane norgestimate;(2)cofactors' role in progesterone-associated anaphylaxis (aeroallergens, alcohol, exercise);(3)progesterone serum concentration's relevance in clinical reactivity, with recurrent reactions occurring at maximal concentration after transdermal application. Exogenous progesterone intakes must be considered in the diagnosis.

Learning objectives

1. To describe the occurrence of IgE- and non-IgE-mediated hypersensitivity symptoms to progesterone.
2. To highlight the modes of investigation of an exogenous progesterone hypersensitivity reaction
3. To establish and select appropriate therapeutic modalities in progesterone hypersensitivity in women of childbearing age.
4. To learn more about progesterone hypersensitivity's various clinical presentations and management.

Takeaway Message

Management of PH in sexually active women of reproductive age remains a challenge with the rising use of contraceptive methods. It has been associated with endogenous as well as exogenous sources of progestogens and can evolve over time.