The Third
Skin@Bath Network Symposium

14th–16th December 2022

Guildhall, Bath, UK

Abstract Book

Skin@Bath Network Symposium 2022

Welcome Message

Dear Friends,

On behalf of the members of the Organising Committees we would like to welcome you to the "Second Skin@Bath Network Symposiun".

We have been overwhelmed by the number of delegates, both early-career and more established clinical and non-clinical scientists and physicians, who have registered for this meeting and have expressed a willingness to give oral or poster presentations. We also appreciate the encouragement you have given us to organise what we hope is a meeting that brings together clinical and non-clinical scientists from many different disciplines and research areas, all of whom have interests in the skin.

Many of you have travelled long distances to attend this meeting in the historic city of Bath. Some of you are visiting Bath for the first time whereas others have been fortunate to visit the city before. Although we have a very intensive scientific programme over the next two days, we have also been able to organise a reception dinner on the 15th which we trust will provide a relaxing networking opportunity at what is a particularly special time of the year in the run-up to the Festive period.

We trust that during the meeting you will have plenty of opportunities not only to renew old friendships with long-term collaborators but also to establish new collaborations. We also hope that in the future we will be able to look back at this meeting in Bath as the third one of a regular series of Symposiun devoted to skin.

We wish you a fruitful and pleasant stay in Bath.

Best wishes,

Charareh Pourzand, Caoimhe Fahy, Celia Mead, Francesca Staples

SKIN@BATH 2022 Acknowlegments

The Skin@Bath organising committee gratefully acknowledges the valuable support from:

PFIZER

SANOFI

ABBVIE

JANSENN /JOHNSON & JOHNSON

BLUESIDE PHOTONICS/ COLORDYNE LTD

(As part of The Centre For Therapeutic Innovation Hub, University of Bath)

Skin@Bath Program

Wednesday 14th December 2022

19.00-20.00 **Welcome Drink Reception** *Venue: Victoria Art Gallery Museum, Bath*

(For registered attendees only; Not for sponsors)

****

Thursday 15th December 2022 Venue: Guildhall, Bath

8:00-8:30 **Registration and Coffee / Tea Break** (Guildhall foyer)

8:30- 8:50 **Welcome Speeches** (Banqueting room) [*Chair:* ***Chris Lovell***]

8:30 **Caoimhe Fahy** and **Charareh Pourzand** [5 min]

8:35 **Adele Murrell**, Head of Life Sciences Department, University of Bath [5 min]

8:40 **Banafshe Larijani**, Director of Centre for Therapeutic Innovation [5 min]

8:45 **Celia Mead**, Director of Bath Institute for Rheumatic Disease, Nick Hall memorial [5 min]

8:50-9:20 **Short Oral Presentations (SOP) I** (Banqueting room)

[Chair: ***Nikoletta Fotaki****]*

8:50 **The use of confocal Raman as a non-invasive method to assess drug pharmacokinetics and bioavailability in the skin.** [10 min]

Panagiota Zarmpi*, Department of Life Sciences, University of Bath, Bath, UK*

9:00 **Biosensing -Micro/nano- scale devices for monitoring and drug delivery applications** [10 min]

Ghazal Hatami-Fard*, The Hamlyn Centre, Imperial College London, London, UK*

9:10 **Investigating RHO kinase inhibition of epidermal stem cells: The promise of infinite skin graft generation?** [10 min]

Benjamin P. Flynn*, Department of Life Sciences, University of Bath, Bath, UK*

9:20-09:45 Keynote speaker I (Guildhall-Banqueting room) [***Chair:*** *Rex Tyrrell]*

MELANIN, DNA PHOTODAMAGE AND VITAMIN D SYNTHESIS [25 min]

Antony R Young*, St John's Institute of Dermatology, King's College London, London, UK*

**********09:45-10:10 Coffee / Tea Break**********

10:10-11:40 Session I: Skin aging and photo-aging [***Chair****: Rex Tyrrell*]

10:10 **The impact of the menopause on skin function** [25 min]

Rachel Watson*, University of Manchester, UK*

10:35 **UVR-induced degradation of dermal extracellular matrix and protection by green tea catechins** [15 min]

Mark Farrar, *University of Manchester, UK*

10:50 **Sunscreens: Study of Irritation and Permeation** [15 min]

Karl Lawrence, *Keratify, UK*

11:05 **Antioxidant function of a key metabolic integrator coenzyme A in cellular responses to oxidative or metabolic stress** [15 min]

Ivan Gout, *University College London, London, UK*

11:20 **Targeting cell signalling regulation in skin aging and photoaging** [20 min]

Pornngarm Dejkriengkraikul, *Chiang Mai University, Chiang Mai, Thailand*

11:40-12:05 State of the Art Lecture I (Banqueting room) [***Chair :*** *Ioav Cabantchik]*

The Fusion of Quantitative Molecular Proteomics and Immune-oncology: A Step towards Precision Medicine in Cancer Therapeutics [25 min]

Banafshe Larijani, *Centre for Therapeutic Innovation, University of Bath, UK*

******12:05-14:05 Lunch Break & Poster Presentation******

Lunchtime Session [12:45-13:55] (Kaposvar room)

[Chairs: ***Chris Lovell & Richard Guy***]

12:45 NIHR Clinical Research Network - Supporting Dermatology Research [20 min]

Hannah Williams, Greater Bristol Area, United Kingdom

13:05 Research with impact: Transdermal Diagnostics - a University of Bath spin-out [20 min] ***Richard Guy***, *Department of Life Sciences, University of Bath, Bath, UK*

13:25 ***The use of teledermatology around the world and in a tertiary centre in the UK*** [20 min]
Charan Singh Thandi, *University Hospitals Bristol and Weston NHS Foundation Trust,UK*

13:45 Combination of cold atmospheric plasma and biomimetic membrane as a versatile toolbox in redox medicine [10 min]
Mehdi Ravandeh, *Leibniz Institute for Plasma Science and Technology, Greifswald, Germany*

14:05-15:20 Session IIa: Skin melanoma cancer (Banqueting room)

[Chairs: ***Ute Jungwirth*** & ***Keith Vance***]

14:05 **Current Melanoma Treatments: An overview** [30 min]

Mark Middleton, *University of Oxford & Cancer Research UK Oxford, UK*

14:35 **Phenotypic heterogeneity in melanoma** [30 min]

Colin Goding, *Ludwig Cancer Research, University of Oxford, UK*

15:05 **MITF-SOX10 regulated long non-coding RNAs in melanoma** [15 min]

Keith Vance, *Department of Life Sciences, University of Bath, UK*

******15:20-15:45 Coffee/Tea Break******

15:45-17:05 Session IIb: Skin non-melanoma cancer (Banqueting room)

[**Chairs:** *Ute Jungwirth & Gernot walko*]

15:45 **Current treatments for basal cell carcinoma: An Overview** [25 min]

Girish Patel, *Welsh Institute of Dermatology, University Hospital of Wales & Cardiff Univ, UK*

16:10 **The Roles of YAP/TAZ and the Hippo Pathway in non melanoma skin cancer**

[15 min] ***Gernot Walko,*** *Department of Life Sciences, University of Bath, UK*

16:25 ***The role of*** **microRNAs in skin cancer**

[20 min] ***Pinar Uysal Onganer***, *Department of Life Sciences, University of Westminster, UK*

16:45 **Importance of tissue-relevant oxygen concentrations when testing cytotoxic redox-active drugs in cultured human skin cancer cells** [20 min]

Paul Winyard*, University of Exeter Medical School , Exeter, UK*

17:05-17:30 Keynote speaker II (Banqueting room) [**Chair:** *Rex Tyrrell*]

Ethnic Skin photoprotection [25 min]

Paul Matts*, Procter & Gamble, UK*

17:30-18:00 Welcome note: (Banqueting room)

[**Chairs :** *Caoimhe Fahy and Charareh Pourzand*]

Prof Ian White, *Vice Chancellor, University of Bath*

18:00-19:30 Drinks Reception (Guildhall, Brunswick room)

19:30-23:00 Banquet dinner (Guildhall, Banqueting room)

Social events: *(For registered attendees only; Not for sponsors)*

****

Friday 16th December 2022 (Guildhall, Bath)

8:00-8:15 Registration and Coffee / Tea Break (Guildhall foyer)

8:15-8:45 Short Oral Presentations (SOP) II [(Banqueting room)

*[**Chair:** Faiza Benaouda]*

8:15 Selected SOP - **Preventing cutaneous sensitization to allergenic proteins using a novel barrier cream**, (10 min)

Sumayah Abdul-Jabbar*, King's College London, London, UK*

8:25 Selected SOP - **Role of epidermal hepcidin in Psoriasis**, (10 min)

Elise Abboud*, Université Paris Cité, CNRS UMR8104, INSERM U1016, Institut Cochin, Paris, France*

8:35 Selected SOP - **Reverse iontophoresis to predict topical bioavailability** (10 min)

Kieran Moore*, Department of Life Sciences, University of Bath, Bath, UK*

8:45-9:10 Keynote speaker III (Banqueting room) [***Chair:*** *Ioav Cabantchik]*

Skin Sensory Dysfunction Produced by Chemotherapeutic drugs [25 min]

Antonio Vicente Ferrer Montiel*, Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universitas Miguel Hernández, Elche, Spain.*

9:10-9:35 State of the Art Lecture II (Guildhall-Banqueting room) [***Chair:*** *Ioav Cabantchik]*

Cold Physical Plasma as a Clinical Device and Biomedical research tool. [25 min]

Kristian Wende*, Leibniz Institute for Plasma Science and Technology (INP Greifswald), Center for Innovation Competence (ZIK) plasmatis, Greifswald Germany*

********09:35-10:00 Coffee/Tea Break********

10:00 -11:00 Session III: Skin and Inflammation (Banqueting room)

[Chairs: Chris Lovell and Steve Ward]

10:00 **Predicting Psoriatic Arthritis** [20 min]

Neil McHugh, *Department of Life Sciences, Centre for Therapeutic Innovation, University of Bath, UK*

10:20 **The Psoriatic arthritis: Treatment options with biologic and targeted synthetic DMARDs** [20 min]

Will Tillett, *Department of Life Sciences, Centre for Therapeutic Innovation, University of Bath; Royal National Hospital for Rheumatic Diseases, Bath, UK*

10:40 ***Sponsored talk*****- Psoriasis and atopic dermatitis: two sides of a coin?** [20 min]

Paola Di Meglio*, King's College London, UK* ***& Josie Evans,*** *Medical Science Liaison, Dermatology, Sanofi, UK*

11:00-11:45 Session IV: Skin and Covid19 (Banqueting room)

[*Chair:* ***Stuart Jones***]

11:00 **Covid 19, iron and the complexity of intertalk between the host and SARS-CoV-2 virus** [25 min]

Marvin Edeas, *Université de Paris, INSERM U1016; Institut Cochin, CNRS UMR 8104; Laboratory of Excellence GR-Ex, Paris, France.*

11:25 **Photoinactivation of coronaviruses with far UVC** [20 min]

Michael Conneely*, University of Dundee, Dundee, UK.*

11:45-12:15 Keynote speaker IV (Banqueting room) [***Chair:*** *Ioav Cabantchik*]

Iron sensing and chelation for adjustment and removal of excess iron in health and diseases [30 min]

Robert Hider*, King's College London, London, UK*

******12:15-14:15 Lunch Break & Poster Presentation******

Parallel Lunchtime session II [13:10-14:05] (Banquetting room)

[**Chair**: *Chris Lovell*]

13:10 **Differences between adult and juvenile onset dermatomyositis including rash, calcinosis** *[20 min]*

Sarah Tansley*, Department of Life Sciences, Centre for Therapeutic Innovation, University of Bath, UK*

13:30 **Daylight PDT in Scotland** *[20 min]*

Marese O'Reilly*, Department of Photodermatology, University of Dundee, Dundee, UK*

13:50 **Smart implanted access port catheter for cancer therapy intervention with pH and lactate biosensors** [15 min]

Salzitsa Anastasova*, Imperial College London, UK*

Parallel Lunchtime session III ((China-UK Skin Consortium – Dermatology, Skin and Biomechanics/Biomedical Engineering Research) [13:10-14:10] (Kaposvar room)

[Chair: Rex Tyrrell]

13:10 AI in Skin: Standardizing the management of psoriasis in China [15 min] ***Hang LI,*** Dermatology Departmentt, The First Affiliated Hospital of Beijing University, Beijing, China.

13:25 Novel therapeutic strategy in immunotherapy of melanoma

[10 min] ***Hong LIU,*** Dermatology Dept., Xiangya -the First Affiliated Hospital of Central South University, Changsa, China

13:35 Tissue Mechanics in Haired Murine Skin: Potential Implications for Skin Aging AI in Skin: Standardizing the management of psoriasis in China
[10 min] ***Mingxing Lei,*** 111 Project Laboratory of Biomechanics and Tissue Repair; Key Laboratory of Biorheological Science and Technology of the Ministry of Education, College of Bioengineering, Chongqing University, Chongqing, China.

13:45 Photobiology in our eye: A prospective [10 min]
Juan Su & Julia Li Zhong, Dermatology Dept., Xiangya -the First Affiliated Hospital of Central South University, Changsa; 111 Project Laboratory of Biomechanics and Tissue Repair; Key Laboratory of Biorheological Science and Technology of the Ministry of Education College of Bioengineering, Chongqing University, Chongqing, China.

13:55 Why red-haired individuals are so prone to developing melanoma [15 min]
Rutao CUI, Medical School, Zhejiang University, Hangzhou, China

14:15-15:55 Session V: Skin models of diseases and tissue regeneration

(Banqueting room) [**Chairs:** *Sara Anjomani-Virmouni and David Gurevich***]**

14:15 **Fetal cells to help skin wound repair** [25 min]

Selim Aractingi*, Université Paris Cité, CNRS, INSERM, Institut Cochin, Paris; Service de Dermatologie, Hôpital Cochin, Assistance Publique-Hôpitaux de Paris, France.*

14:40 **Improve an old poison to treat profibrotic skin diseases** [20 min]

Carole Nicco*, Université Paris Descartes, Sorbonne Paris Cité, INSERM U1016, Institut Cochin, CNRS UMR8104, Paris, France; BioSenic, Louvain La Neuve, Belgium.*

15:00 **Molecular mechanisms of melanin transfer and processing** [20 min]

Duarte Barral, *Chronic Diseases Research Center (CEDOC), NOVA Medical School, NMS, Universidade NOVA de Lisboa, Lisbon, Portugal.*

15:20 **Epidermal hepcidin in infectious and inflammatory diseases** [20 min]

Carole Peysonnaux, Université de Paris, Institut Cochin, INSERM, CNRS, F-75014 Paris; Laboratory of Excellence GR-Ex, Paris, France.

15:40 **Fluorescence-activated cell sorting and quantitative Real-Time PCR to reveal VEGF-expressing macrophage populations in the Zebrafish Larvae** [15 min]

David Gurevich*, Department of Life Sciences, Centre for Therapeutic Innovation, University of Bath, Bath, UK*

******15:55 - 16:20 Coffee/Tea Break******

16:20-17:15 Session VI: Skin delivery methods and measurements

(Banqueting room) [**Chair:** Nikoletta Fotaki**]**

16:20 **Stretching the skin to facilitate needleless delivery of vaccines** [20 min]

***Faiza Benaouda**, Institute of Pharmaceutical Science, Faculty of Life Sciences & Medicine, King's College London, London, UK*

16:40 **Understanding vitamin D supplementation; effects of race and delivery route** [20 min]

***Stuart Jones,** Institute of Pharmaceutical Science, Faculty of Life Sciences & Medicine, King's College London, London, UK*

17:00- 17:30 Final Speeches (Banqueting room) [***Chairs:** C. Fahy and C. Pourzand*]

Final Acknowledgements/ Best Poster & Oral Presentation Prize Draw.

Sponsors: Pharmaceutical companies have provided funding for this event. This event is operated independently. Funding will not be used for any social activities within the programme.

Oral Presentations

O1

THE USE OF CONFOCAL RAMAN AS A NON-INVASIVE METHOD TO ASSESS DRUG PHARMACOKINETICS AND BIOAVAILABILITY IN THE SKIN.

P. Zarmpi[1], D. Tsikritsis[2], A. Watson[1], J-L. Vorng,[2], V. Tyagi[2], P. Ghosh[3], N.A. Belsey[2], T.J. Woodman[1], K.A.J. White[1], A.L. Bunge[4], M.B. Delgado-Charro[1], R.H. Guy[1]

[1]University of Bath, U.K.; [2]National Physical Laboratory, U.K.; [3] Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, U.S.A; [4]Colorado School of Mines, U.S.A.

pz300@bath.ac.uk

Th The objective of the study is to demonstrate that Raman spectroscopy can characterise the epidermal bioavailability of a topically applied drug and correctly distinguish formulations that are expected to be bioequivalent from those that are not. The study was conducted using two approved metronidazole (MTZ) topical gels that are known to be bioequivalent and two laboratory-made solutions of the drug.

The formulations used were (a) fully saturated MTZ solutions in 90:10 and 30:70 v/v water/propylene glycol (PG), and (b) 0.75% w/w MTZ gels manufactured by Prasco® and Tolmar®. Drug uptake after 6- and 12-hour applications, and clearance 2 and 4 hours later, of each of the 4 formulations were assessed *ex vivo* using abdominal pig skin (4 replicates each on tissue from 3 animals), without occlusion. Raman spectra were acquired with a Renishaw inVia microscope using a 785 nm laser. The C=N signal (at 1192 cm^{-1}) from MTZ enabled detection of the drug as a function of depth; this vibration provided a strong intensity distinct from the background skin spectrum. The permeation of PG (an inactive ingredient in all formulations studied) was also monitored via its characteristic signal at 840 cm^{-1}. Signal attenuation was accounted for by normalising drug or inactive ingredient intensities to the corresponding amide I signal in each spectrum. Depth profiles acquired from untreated skin confirmed the absence of interference at the frequencies of interest.

The Raman-deduced disposition of MTZ from the gels appeared to be consistent as a function of time and depth into the skin – both for a within-gel comparison of product manufactured by Prasco®, and comparison of the two gels. In contrast, the composition of the two solutions clearly altered the cutaneous pharmacokinetics of MTZ. A possible explanation for the differences in MTZ disposition when applied as solutions with different water/PG ratios is the rapid metamorphosis of the 90:10 water/PG MTZ solution and precipitation of MTZ.

These qualitative observations will be supplemented by extracting appropriate metrics to quantify topical drug bioavailability and to determine bioequivalence (or not) between the products assessed.

O2

BIOSENSING -MICRO/NANO- SCALE DEVICES FOR MONITORING AND DRUG DELIVERY APPLICATIONS

Ghazal Hatami-Fard, Salzitsa Anastasova-Ivanova†

The Hamlyn Centre, Imperial College London, South Kensington Campus, London SW7 2AZ, UK

Sensing science has had a huge impact on facilitating daily life and further on the health care and diagnostics industry. Electrochemical biosensors are capable to be designed and characterised to be utilised in various biomedical concepts. So far the development of advanced small-sized biosensors was perfectly successful in initiating their way into the diagnostics and prevention market. Applications include cell-specific drug delivery via microbots (Anastasova et al., 2010), biosensing of specific analytes (Calcium, potassium, proteins, etc) via wearable sensors (Anastasova et al., 2017), and continuous monitoring of analytes via semi-implantable biosensors (Anastasova et al., 2020) such as subdermal glucose sensing microneedles are yet a few instances in this area. Development techniques for each system are different, As in the Micro/Nano-scale devices can be developed via 3D printing and functionalised via electrochemical methods by characterising suitable substrates. Whereas electrochemical biosensing platforms can be prepared via suitable characterisation of a wire/metal/gel-based substrate to detect an analyte of interest depending on the ultimate application (Gil et al., 2020). Our research focuses on the fabrication of functional wearable/semi-implantable biosensors and microrobots for early-onset detection, diagnosis, screening, and cell-specific drug delivery for notorious diseases such as cancer.

References

- Anastasova, Salzitsa, et al. "A wearable multisensing patch for continuous sweat monitoring." Biosensors and Bioelectronics 93 (2017): 139-145
- Anastasova-Ivanova, Salzitsa, et al. "Development of miniature all-solid-state potentiometric sensing system." Sensors and Actuators B: Chemical 146.1 (2010): 199-205.
- Anastasova, Salzitsa, et al. "Electrochemical monitoring of subcutaneous tissue pO2 fluctuations during exercise using a semi-implantable needle electrode." Electroanalysis 32.11 (2020): 2393-2403.
- Gil, Bruno, Salzitsa Anastasova, and Benny Lo. "Graphene field-effect transistors array for detection of liquid conductivities in the physiological range through novel time-multiplexed impedance measurements." Carbon 193 (2022): 394-403.

O3

INVESTIGATING RHO KINASE INHIBITION OF EPIDERMAL STEM CELLS:

THE PROMISE OF INFINITE SKIN GRAFT GENERATION?

Benjamin P. Flynn, Kelli Gallacher & Gernot Walko

Department of Life Sciences, University of Bath, Claverton Down, Bath, BA2 7AY, UK

Premature depletion of self-renewing holoclone-forming epidermal stem cells (epSCs) within autologous human keratinocyte cultures is a major complication in generation of epidermal transplants for treatment of skin defects such as burn wounds. Senescence occurs when the last colony producing epSC present within keratinocyte cell culture loses its stemness and generates abortive paraclone colonies, a process referred to as clonal conversion. Rho kinase inhibitor treatment (ROCKi) has been shown to enable conditional 'immortalisation' of human keratinocyte cultures(1,2), thus holding great promise for regenerative medicine applications. However, the signalling mechanisms involved in conditional immortalisation by continuous ROCKi treatment, and the downstream transcriptomic effects, remain poorly understood. It also uknown if ROCKi-treatment stabilizes the epSC state in keratinocyte culture, or drives conversion into highly proliferative transit-amplifying progenitor cells with reduced tissue-regenerating capacity. To this end, we investigated RNA-Seq data generated from (i) early passage human keratinocytes (passage 5), (ii) late passage culture with depleted SC pool and (iii) ROCKitreated, highly proliferative late passage culture (passage 8). Colony formation assays were used to evaluate the effects of ROCKi and its removal (> passage 25). Our bioinformatic analysis shows that gene expression signatures associated with the epSC state (holoclones) become highly enriched in response to ROCKi treatment. However, cell culture experiments indicate that the ROCKi-induced epSC state is conditional and unstable, as cells rapidly commit to terminal differentiation upon ROCKi withdrawal, possibly because replicative memory is not erased by ROCKi treatment.

As we expect pharmacological inhibition of ROCKi to cause highly pleiotropic downstream effects, further investigation is required to identify the specific signalling cascades that are deregulated and how this causes conditional programming of the epSC state. Summarised, the observations we have made so far caution against the use of ROCKi for the generation of human epidermis transplants.

1. Peters-Hall , J., et al. (2020) FASEB journal. 34:386-398
2. Chapman, S., (2014) Stem Cell Research and Therapy. 5:60

O4

MELANIN, DNA PHOTODAMAGE AND VITAMIN D SYNTHESIS

Antony R Young

St John's Institute of Dermatology; King's College London, London, UK

There has been limited research on the photobiology of pigmented skin. Epidemiology informs us that skin cancer risk is very low in those with black skin, and that vitamin D status is sub-optimal. The role of melanin has been largely inferred rather than experimentally determined. The St John's experimental photobiology team has quantified the benefits of melanin on DNA photodamage as a measure of reducing risk, and on possible inhibition of vitamin D synthesis that is a loss of benefit. The marker of DNA damage was the thymine dimer (T<>T) and vitamin D status was assessed by serum 25(OH)D_3. Action spectra for both endpoints for skin *in vivo* peak in the UVB region. The approach was to compare young healthy volunteers of Fitzpatrick skin (FST) type I/II with VI, after exposure to solar simulating radiation. Melanin was shown to be highly protective against T<>T, in the basal layer of the epidermis (DNA protection factor of ~60) but much less so in the suprabasal layers. This is important because the basal layer contains keratinocyte stem cells and melanocytes. Dark or delayed T<>T, that occur 2-3 hours post-UVR exposure and have been attributed to chemiexcitation of melanin intermediates, were observed in FST I/II and VI. In contrast to being highly effective at inhibiting basal layer DNA damage, melanin had a very modest effect on vitamin D synthesis, with an inhibition factor of <1.5. The most likely reason for different effects of melanin is the melanin concentration gradient with epidermal depth. A very high basal layer concentration protects cells in this zone from DNA damage. The precursor (chromophore) for vitamin D is 7-dehydrocholesterol which is abundant in suprabasal layers where melanin concentration is relatively low. These results suggest that the low incidence of skin cancer in FST VI is the consequence of high levels of melanin protection in the basal layer, and that sub-optimal vitamin D status can be improved by increased solar exposure without any significant increased risk of skin cancer. They also stress that studies on melanin must be done in the context of its spatial relationship to the target chromophore.

O5

ONEVIVO: A NOVEL *EX VIVO* SKIN CULTURE DEVICE ALLOWS CLINICALLY RELEVANT SKIN TOXICOLOGY TESTING

K P. Lawrence[1,2], C-P. Kung[2], S. Bashall[1], E. McPolin-Hall[2], I. Barnard, , S. Brand[3], G. Georgeu[3], Z. Ahmed[2], J. Frame[3], M P. Philpott[1], J R. Castrejón-Pita[4], R F. Hannen[1,2*]

[1] *Centre for Cell Biology & Cutaneous Research, Blizard Institute, Queen Mary University of London, E1 2AT*
[2] *Keratify Ltd, 4 Newark Street, London, E1 2AT*
[3] *Anglia Ruskin University, Chelmsford, Essex, CM1 1SQ*
[4] *School of Engineering and Materials Science, Queen Mary University of London, London, E1 4NS.*

The skin is a formidable barrier that protects the body from the external environment. Unfortunately, crucial physiological characteristics of healthy skin are lost with standard tissue culture techniques, where skin barrier competency is poorly replicated (3D skin models) or maintained (*ex vivo* skin models). Furthermore, healthy skin tissue and cells typically exhibit stressed, semi-wounded or partial-disease responses as a consequence of culture. As a result, current skin testing practices routinely require separate laboratory tests for dermal absorption, corrosion, irritation, sensitisation and biological efficacy with predetermined time points. Sunscreens are widely used cosmetic formulations containing UV filters and other ingredients that protect the skin from excessive UV exposure. Regulated as cosmetics, these compounds are required to undergo regulatory approved toxicology testing. In recent years, increasing amounts of data has been generated to suggest that these filters may not be as safe as first thought, suggesting current toxicology testing to be inadequate. These concerns have raised the attention of a number of regulatory bodies with some filters at risk of a ban entirely. Here we examine how to resolve dermatology testing limitations and simultaneously improve the speed of testing through engineering, computational analysis and biological consideration. We engineered a biphasic fluidic culture device utilising atmospheric air, pH control and media flow control to mimic the physiological environment to support *ex vivo* skin. This resulted in high fidelity maintenance of *ex vivo* skin barrier and tissue function, with skin structure, cell viability, transcutaneous electrical impedance and key differentiation markers maintained for at least 5 days. Conversely, other standard culture techniques (Franz cell or standard tissue culture incubator) led to significant skin tissue degradation from 24 hours. Further analysis demonstrated our technology enhanced skin toxicology assessment to better mimic human responses. This included difficult to categorise detergents and also correctly predicted the irritation classification of select OECD validation compounds. Inclusion of new semi-automated skin surface analysis additionally enabled real-time assessment of skin surface properties. Using non-invasive techniques, we were able to provide continuous monitoring into the 4th dimension, to ultimately eliminate the need for pre-determined experimental time points. Collectively, this system offers a new tool for high-fidelity preclinical skin testing to improve safety assessment of dermatology products.

O6

ANTIOXIDANT FUNCTION OF A KEY METABOLIC INTEGRATOR COENZYME A IN CELLULAR RESPONSES TO OXIDATIVE OR METABOLIC STRESS.

Ivan Gout

Department of Structural and Molecular Biology, University College London, London, UK.
Institute of Molecular Biology and Genetics, Kyiv, Ukraine.

Coenzyme A (CoA) is a key metabolic cofactor in all living cells. CoA and its thioester derivatives (acetyl-CoA, malonyl-CoA, HMG-CoA etc.) participate in diverse anabolic and catabolic pathways, allosteric interactions, biosynthesis of neurotransmitters and the regulation of gene expression. Dysregulation of CoA/CoA derivatives biosynthesis and homeostasis has been associated with various human pathologies, including cancer and neurodegeneration and metabolic disorders.

We have recently discovered a novel mode of redox regulation, involving covalent modification of cellular proteins by CoA (protein CoAlation) in cellular response to oxidative or metabolic stress. To discover and study this novel post-translational modification, we have developed several novel reagents and methodologies, including: (a) anti-CoA mAb, which specifically recognize CoA in ELISA, WB, IP and IHC (no anti-CoA antibodies are commercially available); (b) a robust mass spectrometry-based methodology for the identification of CoAlated proteins; and (c) efficient *in vitro* CoAlation and deCoAlation assays. Cell-based and animal models have been employed to demonstrate that protein CoAlation is a reversible and widespread post-translational modification induced by oxidizing agents and metabolic stress in prokaryotic and eukaryotic cells. To date, we have identified using the developed methodology more than 2100 proteins, which are CoAlated under various experimental conditions in cell and tissues. We showed that protein CoAlation modulates the activity and subcellular localization of modified proteins. It can also protect oxidized cysteine residues from overoxidation and induce significant conformational changes. We have employed biochemical, biophysical, crystallographic and cellular approaches to study the mode of CoA binding to a panel of selected metabolic enzymes and signalling proteins. Over the last 5 years we have firmly established ourselves as a world-leading laboratory on protein CoAlation and the antioxidant function of CoA in health and pathologies associated with oxidative stress, including cancer and neurodegeneration. Based on these findings, we propose that under physiological conditions CoA functions to produce metabolically-active derivatives, but acts as an antioxidant in cellular response to oxidative or metabolic stress.

Human skin is exposed to a diverse range of harmful agents which can lead to oxidative damage in DNA, proteins and lipids caused by reactive oxygen species (ROS). This conference is an excellent opportunity for gain the knowledge on the pathogenesis of various skin pathologies and to discuss the prospects of collaborative interactions on the emerging topic of the antioxidant function of CoA.

O7

TARGETING CELL SIGNALLING REGULATION IN SKIN AGING AND PHOTO-AGING

Pornngarm Dejkriengkraikul

Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Thailand. Center for Research and development of natural products for Health, Chiang Mai University, Thailand.

UV radiation is a major exogenous causing factor for many cutaneous diseases including skin damage, aging, inflammation, wrinkling and melanoma. Understanding the modulation in cell signaling pathways induced by UVA and UVB, new strategic era for skin prevention and treatment of UV-related diseases could be improved. The direct DNA damage and ROS production caused by skin exposure to solar UV radiation lead to activation of cytoplasmic signal transduction pathways, which are related to cell survival, death and inflammation. The loss of intracellular redox balance in the skin during UV-mediated oxidative stress results in cellular damage and eventually leads to intrinsic and extrinsic apoptotic cell death and over-production of proinflammatory mediators such as IL-1α, IL-18, IL-6, IL-8 and TNF. Differential effects of UVA and UVB on the activation of PI3K/Akt and mitogen-activated protein kinases (MAPKs) pathways including p38, ERK and JNK might explain target interventions against signaling events mediated by UV exposure. Targeting skin aging with phytochemicals as potential prodrugs has emerging in the spotlight in very recent years. Many of these phytochemicals are able to adapt the ROS signaling to modulate the cytoplasmic signaling pathways as aforementioned targeted signal pathways in a culture skin model of UVB-irridation. These signal transductions activate the function of AP-1 nuclear transcription factor, resulting in the inhibition of collagen production and increase the number of matrix metalloproteinase to decompose collagen, which eventually leads to skin aging. Examples of our previous research findings, many polyphenol-containing plants were found to ameliorate oxidative stress and block the skin aging-related pathways for both keratinocytes and fibroblasts skin models. Furthermore, we are now developing the transcriptomics platform of those targeted genes that are modulate by UVB-irradiation in skin keratinocytes and fibroblasts. The powerful tool of pathway enrichment analysis (PEA) or gene set enrichment analysis (GSEA) should help us for understanding the biology underlying the data contained in metadata of differentially expressed genes (DEGs), resulting from modern high-throughput profiling technologies and pave the way to novel molecular mechanism in skin aging paradigm.

O8

THE FUSION OF QUANTITATIVE MOLECULAR PROTEOMICS AND IMMUNE-ONCOLOGY: A STEP TOWARDS PRECISION MEDICINE IN CANCER THERAPEUTICS

Banafshé Larijani

Centre for Therapeutic Innovation, Cell Biophysics Laboratory, Department of Life Sciences,, University of Bath, Claverton Down, Bath, BA2 7AY United Kingdom

I will be presenting how we have employed, in an interdisciplinary arena, quantitative molecular biology to investigate *in situ* molecular mechanisms in the PI 3Kinase pathway. These studies led to applying our molecular imaging methods in clinical environments. We have addressed pertinent questions regarding the exploitation of functional proteomics, as opposed to expressional proteomics in diagnostics and prognostics of various types of cancers.
The implementation of this type of quantitative imaging, in deciphering mechanisms involved in signalling pathways, has led to exploiting spatio-temporal interactions of the immune-checkpoint proteins, and their functionally defined as biomarkers for determining whether interactions blocking immunotherapies are appropriate treatments in cancer patients.

O9

COMBINATION OF COLD ATMOSPHERIC PLASMA AND BIOMIMETIC MEMBRANE AS A VERSATILE TOOLBOX IN REDOX MEDICINE

Mehdi Ravandeh[1], Heike Kahlert[2], Maria Fedorova[3], Kristian Wende[1]

[1]*Leibniz-Institute for Plasma Science and Technology, Germany*

[2]*Institute of Biochemistry, University of Greifswald, Germany*

[3]*Center of Membrane Biochemistry and Lipid Research, University Hospital and Faculty of Medicine Carl Gustav Carus of TU Dresden, Germany*

Cold atmospheric plasma (CAP) is an emerging technology that is being used for biomedical purposes like wound healing, treatment of skin related disorders, and cancer [1,2]. In the last decade, biomimetic membranes such as lipid vesicles and supported lipid bilayers (SLB) have been developed to simplify the complexity of the cell membrane. In this study, we combined CAP and SLB to investigate the protective role of sphingomyelin (SM) in the eye lens membrane during aging using analytical techniques such as electrochemistry, high-resolution mass spectrometry (HR-MS) and atomic force microscopy (AFM). Cataracts, as an age-related disease, can result from ultraviolet radiation and oxidative damage in the eye lens during aging[3]. In the eye lens cell membrane, the lipid composition changes during the aging process: the proportion of sphingomyelins (SM) increases while that of phosphatidylcholines decreases[4]. Here, Supported lipid bilayers (SLB) were prepared to mimic the lens membrane with different fractions of PC/SM. Cold atmospheric plasma was used to generate hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($^{\bullet}O_2^-$), and H_2O_2, since these ROS reportedly contribute to cataract formation. After 30 min plasma treatment of SLB, a protective effect of 29% and 46% was obtained in the presence of 25% and 75% SM in the bilayer, respectively. Lipid oxidation products were determined via HR-MS for plasma-treated SLBs. Topographic images obtained by AFM of PC-bilayers showed degradation and pore formation after plasma treatment, no degradation was observed in PC/SM bilayers. The results confirm the protective role of SM and support the idea that the SM content in lens cell membrane increases during aging to protect the eye from oxidative damage in the absence of effective antioxidant systems and to prolong lens transparency[5].

1 Ravandeh, M. *et al.* A combination of electrochemistry and mass spectrometry to monitor the interaction of reactive species with supported lipid bilayers. *Sci Rep* **10**, 18683, doi:10.1038/s41598-020-75514-7 (2020).

2 Metelmann, H.-R., Von Woedtke, T. & Weltmann, K.-D. *Comprehensive clinical plasma medicine: cold physical plasma for medical application*. (Springer, 2018).

3 Shichi, H. Cataract formation and prevention. *Expert Opin Inv Drug* **13**, 691-701, doi:10.1517/13543784.13.6.691 (2004).

4 Perry, R. H., Borchman, D. & Byrdwell, W. C. Changes in the phospholipid composition of the human lens with age and cataract formation. *Investigative Ophthalmology & Visual Science* **44**, U534-U534 (2003).

5 Ravandeh, M. *et al.* Protective Role of Sphingomyelin in Eye Lens Cell Membrane Model against Oxidative Stress. *Biomolecules* **11**, doi:10.3390/biom11020276 (2021).

O10

MITF-SOX10 REGULATED LONG NON-CODING RNAS IN MELANOMA

Stephanie Jones[1], Robert N Kelsh[1] and Keith W Vance[1]

[1] *Department of Life Sciences, University of Bath, Bath, BA2 7AY*

The human genome expresses many thousands of long non-coding RNAs (lncRNAs) that do not code for protein, the majority of which have unknown function. Recent work has implicated lncRNAs as a new class of cancer causing genes. Somatic copy-number alteration of lncRNA loci frequently occurs in different cancer types and lncRNAs are often expressed in a tumour cell specific manner. LncRNAs that function as tumour suppressors or oncogenes have now been identified in melanoma, demonstrating a wide range of critical roles for these molecules in melanoma biology. While melanoma associated lncRNAs have great potential for the development of new melanoma treatments, how lncRNAs integrate into the well-defined gene expression networks that underpin melanoma growth and metastasis is less clear.

The MITF transcription factor is a central regulator of melanocyte development and melanoma biology and acts as a rheostat to govern the generation of different melanoma cell states in response to changes in the microenvironment. MITF regulates the expression of protein coding genes involved in cancer associated processes such as proliferation, differentiation, invasion, DNA replication and repair and cancer metabolism. In this talk, I will present our recent work showing how lncRNAs integrate into the MITF network in melanoma [1] and describe a prioritised subset of clinically relevant MITF-regulated lncRNAs that can control multiple different aspects of melanoma biology. We predict that a switch in focus from the protein coding to the non-coding portion of the genome will represent a key step for the identification of new anti-melanoma therapeutic targets.

[1] Coe, E.A., Tan, J.Y., Shapiro, M., Louphrasitthiphol, P., Bassett, A.R., Marques, A.C., Goding, C.R. and **Vance, K.W.** (2019) The MITF-SOX10 regulated long non-coding RNA *DIRC3* is a melanoma tumour suppressor. *PLosGenetics* **15**(12): e1008501.

O11

CURRENT TREATMENTS FOR BASAL CELL CARCINOMA

Girish Patel

Welsh Institute of Dermatology, University Hospital of Wales & Cardiff University, UK

Basal cell carcinoma (BCC) represents the most common malignancy and as such represents a substantial clinical burden in particular dermatology departments. Usually arising on hair-bearing skin, BCC typically presents as a translucent papule that grows slowly, often over many years, before ulcerating and causing symptomatic spontaneous bleeding. All BCC arise from mutational activation of the hedgehog signalling pathway, with inactivating mutations in the Patched receptors within both basal cell naevus syndrome (90%) and spontaneous (80%) being the most prevalent. The tumours harbour the highest mutational burden, with uniformity of UV signature, yet intriguingly the pathology demonstrates well-organised nests of basal-like keratinocytes invading the dermis and underlying structures. Current management, with the clinical diagnosis being relatively simple, centres on surgical removal; with subtle differences dependent upon the body site. For low-risk sites topical imiquimod, an immune stimulant may be used. While for anatomically constrained sites Mohs micrographic surgery provides the greatest confidence of removal. For inoperable tumours or in the rare instance of metastatic BCC, conventional chemotherapy and immune checkpoint inhibitors have proven ineffective, however, recently introduced smoothened antagonists are effective at reducing tumour burden; albeit often not curative. Our research group has identified hair follicle differentiation within BCC, which appears arrested due to the absence of dermal papilla signals. The tumour growth itself consists of a hierarchical pattern of growth in which growth is dependent upon a small sub-population (up to 3% of tumour cells) of self-renewing BCC keratinocytes, enriched by CD200 protein expression of the cell surface. CD200 is a well-recognised immune checkpoint inhibitor, which is also expressed on normal hair follicle stem cells and therefore may be the cell of origin for BCC. CD200 signalling, through ectodomain shedding, as evident in multiple cancer types results in local immune evasion by blocking the activation of NK cells and leading to apoptosis resulting in a characteristic absence of NK cells in the tumour-invading infiltrate. Blocking CD200 signalling by blocking antibodies led to NK cell activation and tumour cell killing, highlighting a novel approach to BCC treatment.

O12

The Roles of YAP/TAZ and the Hippo Pathway in non-melanoma skin cancer.

Gernot Walko[1,2,3]

[1]Bart's Centre for Squamous Cancer & Centre for Oral Immunobiology and Regenerative Medicine, Institute of Dentistry, Queen Mary University of London, United Kingdom; [2]Department of Life Sciences, University of Bath, United Kingdom; [3]Centre for Therapeutic Innovation, University of Bath, United Kingdom.

Introduction

Nonmelanoma skin cancers (NMSCs) are amongst the most frequently diagnosed human cancers. Cutaneous squamous cell carcinoma (cSCC) accounts for the majority of deaths associated with NMSCs. Like other cancers, cSCC arises from genetic alterations that invariably lead to dysregulated transcriptional programmes. Studies in mice have demonstrated an absolute dependency on the transcriptional co-regulator proteins YAP and TAZ(WWTR1) for cSCC initiation. Most past studies on YAP/TAZ focused on either one of the paralogues or had assumed similar functions between them. However, emerging evidence demonstrates that YAP and TAZ have distinct roles where they partner with different transcription factors, to drive different transcriptional programmes. We have therefore assessed the distinct and redundant roles of YAP and TAZ in a human cSCC cell model.

Materials and methods

Our human cSCC model consists of a panel of isogenic human epidermal cell lines representing different stages of neoplastic progression (MET1, MET2, MET4), which were derived from the clinical progression of a primary human cSCC trough to distant metastasis in a single human immunosuppressed individual. We generated sublines of MET1, MET2 and MET4 expressing YAP- or TAZ-targeting inducible shRNAs to study the functions of YAP and TAZ using variety of 2D and 3D cell culture assays as well as a zebrafish embryo xenograft model.

Results and discussion

We observed variable requirement of YAP and TAZ for cSCC proliferation and survival in 2D and 3D in vitro culture conditions and upon xenografting into zebrafish embryos. Our findings suggest that YAP is the pre-dominant paralogue driving cSCC cell proliferation but appears to cooperate with TAZ during cSCC invasion. However, once cSCC cells have metastasised, they appear to no longer depend on YAP/TAZ for cell proliferation and survival. Interestingly, we observed no obvious correlation between sensitivity to YAP or TAZ depletion and YAP/TAZ expression itself. Thus, our findings strongly argue against the use of YAP/TAZ expression and YAP/TAZ nuclear/cytoplasmic ratio as predictors for YAP/TAZ activity in cSCC. As analysis of YAP/TAZ target gene expression provides a more robust and quantifiable way to assess YAP/TAZ activity than measuring its expression level, subcellular localisation or phosphorylation, our future work will aim to identify a cSCC-specific YAP/TAZ transcriptional signature.

O13

The Roles of YAP/TAZ and the Hippo Pathway in non-melanoma skin cancer.

Gernot Walko[1,2,3]

[1]Bart's Centre for Squamous Cancer & Centre for Oral Immunobiology and Regenerative Medicine, Institute of Dentistry, Queen Mary University of London, United Kingdom; [2]Department of Life Sciences, University of Bath, United Kingdom; [3]Centre for Therapeutic Innovation, University of Bath, United Kingdom.

Introduction

Nonmelanoma skin cancers (NMSCs) are amongst the most frequently diagnosed human cancers. Cutaneous squamous cell carcinoma (cSCC) accounts for the majority of deaths associated with NMSCs. Like other cancers, cSCC arises from genetic alterations that invariably lead to dysregulated transcriptional programmes. Studies in mice have demonstrated an absolute dependency on the transcriptional co-regulator proteins YAP and TAZ(WWTR1) for cSCC initiation. Most past studies on YAP/TAZ focused on either one of the paralogues or had assumed similar functions between them. However, emerging evidence demonstrates that YAP and TAZ have distinct roles where they partner with different transcription factors, to drive different transcriptional programmes. We have therefore assessed the distinct and redundant roles of YAP and TAZ in a human cSCC cell model.

Materials and methods

Our human cSCC model consists of a panel of isogenic human epidermal cell lines representing different stages of neoplastic progression (MET1, MET2, MET4), which were derived from the clinical progression of a primary human cSCC trough to distant metastasis in a single human immunosuppressed individual. We generated sublines of MET1, MET2 and MET4 expressing YAP- or TAZ-targeting inducible shRNAs to study the functions of YAP and TAZ using variety of 2D and 3D cell culture assays as well as a zebrafish embryo xenograft model.

Results and discussion

We observed variable requirement of YAP and TAZ for cSCC proliferation and survival in 2D and 3D in vitro culture conditions and upon xenografting into zebrafish embryos. Our findings suggest that YAP is the pre-dominant paralogue driving cSCC cell proliferation but appears to cooperate with TAZ during cSCC invasion. However, once cSCC cells have metastasised, they appear to no longer depend on YAP/TAZ for cell proliferation and survival. Interestingly, we observed no obvious correlation between sensitivity to YAP or TAZ depletion and YAP/TAZ expression itself. Thus, our findings strongly argue against the use of YAP/TAZ expression and YAP/TAZ nuclear/cytoplasmic ratio as predictors for YAP/TAZ activity in cSCC. As analysis of YAP/TAZ target gene expression provides a more robust and quantifiable way to assess YAP/TAZ activity than measuring its expression level, subcellular localisation or phosphorylation, our future work will aim to identify a cSCC-specific YAP/TAZ transcriptional signature.

O14

THE ROLE OF MICRORNAS IN SKIN CANCER

Pinar Uysal Onganer,

Department of Life Sciences, University of Westminster, UK

microRNAs (miRs) are novel, non-coding RNAs that are often deregulated in human cancers and shown to control cancer related processes of cell growth, differentiation and cell death. miRs can be identified in peripheral blood, saliva, urine, faeces, peritoneal fluid, cerebral-spinal fluid, breast milk, vaginal discharge, semen and pancreatic fluid. Therefore, miRs could be considered as a minimally invasive and easily detected biomarkers for the early diagnosis of cancer. We have identified a panel of miRs that their expressions significantly changed in solid tumours such as prostate, breast, pancreatic adenocarcinoma and recently in cutaneous cell cancer. My presentation will explore the uses of miRs based on our data.

O15

UNDERSTANDING TRANSCUTANEOUS SENSITIZATION TO ALLERGENIC PROTEINS

S. Abdul-Jabbar [1], A Santos [2], R O'Shaughnessy [3], C. Flohr [4], S. A. Jones [1]

[1]*Pharmaceutical Science, King's College London, Faculty of Life Science & Medicine, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom*
[2]*Children's Allergy Service, Evelina London, Guy's and St Thomas' Hospital NHS Foundation Trust, London, UK.*
[3] *QMUL*
[4] *Department Paediatric Allergy, Division of Asthma, Allergy and Lung Biology, King's College London, London, United Kingdom*

Understanding the route and timing of exposure to food allergens in early life has been identified as a research priority. The transcutaneous route of allergen exposure, prior oral consumption, is thought to be important in allergy development [(1)], but how food proteins come into contact with the immune system is currently unknown. As a result of the strong correlation between the prevalence of food allergies and frequency of moisturizing in babies as published by the EAT trial [(2)] this work hypothesised that the allergens enter the skin through hair follicles that have been opened by skin stretching. To test this hypothesis, hydrophilic peanut proteins were extracted from raw peanuts and thermally processed (boiled, roasted, and fried). The extracts were characterised by gel electrophoresis and then labelled fluorescently with Sulfo Cyn5 NHS ester to be able to track their passage through stretched and un-stretched skin. SDS-Page showed that frying modified the levels of Ara h 1 and Ara h 6 proteins. Protein aggregates were present in the roasted peanuts only and displayed a molecular weight of ca. 150 kDa. Preliminary permeation and confocal imaging showed a good indication of proteins entering hair follicles in a stretched skin.

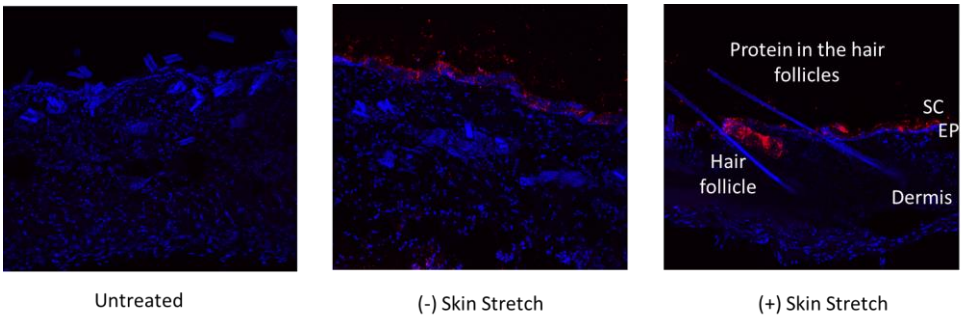


Figure 1. Confocal imaging of a skin cross section after the application of Ara h proteins to the skin with (+) and without (-) the application of skin stretching.

References

1)Lack, G., 2008. Epidemiologic risks for food allergy. Journal of Allergy and Clinical Immunology, 121(6), pp.1331-1336.

2)Perkin, M.R., Logan, K., Marrs, T., Radulovic, S., Craven, J., Boyle, R.J., Chalmers, J.R., Williams, H.C., Versteeg, S.A., Van Ree, R. and Lack, G., 2021. Association of frequent moisturizer use in early infancy with the development of food allergy. Journal of Allergy and Clinical Immunology, 147(3), pp.967-976.

O16

ROLE OF EPIDERMAL HEPCIDIN IN PSORIASIS

Elise Abboud1 , Nadia Boussetta1*, Doha Chrayteh1*, Héloïse Dalle1 , Mariangela Malerba1 , Ting-Di Wu2,3, Charareh Pourzand4 , Joël Poupon5 , Sophie Vaulont1 , Pierre Sohier1,6 , Selim Aractingi1,7 , Zoubida Karim8 , Carole Peyssonnaux1 ** 1

Université Paris Cité, CNRS UMR8104, INSERM U1016, Institut Cochin, F-75014 Paris, France 2 Institut Curie, PSL Research University, Paris, France 3 Paris-Saclay University, CNRS UMS 2016, INSERM US43, Multimodal Imaging Center, Orsay, France 4 Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, UK 5 Laboratoire de Toxicologie Biologique, Hôpital Lariboisière, Paris, France 6 Service de Pathologie, AP-HP, Hôpital Cochin, F-75014 Paris, France 7 Service de Dermatologie, AP-HP, Hôpital Cochin Tarnier, F-75006 Paris, France 8 Toulouse Institute of Infectious and Inflammatory Diseases (Infinity) INSERM UMR1291 - CNRS UMR5051 - Toulouse III University

Psoriasis is an inflammatory skin disease characterized by an abnormally excessive and rapid growth of the epidermal layer of the skin. Several studies extending back to 1973 described an increase of iron in the epidermis of psoriatic patients, suggesting its possible contribution to the hyperproliferation of keratinocytes. On the other hand, neutrophil accumulation within dermis and epidermis is a hallmark feature of psoriasis. Hepcidin, the key regulatory hormone of iron homeostasis, is mainly produced by hepatocytes, but many cells and tissues express this hormone in pathological conditions. We recently found that hepcidin is produced by keratinocytes and play a protective role during bacterial infections triggering CXCL1 production by keratinocytes, the main chemokine for neutrophil recruitment. In this study, we show a dramatic increase of hepcidin in human skin biopsies of psoriatic patients, especially in generalized pustular psoriasis, the acute and severe form of the disease. In mice treated with imiquimod (IMQ), a psoriasis inducer, we also demonstrated an increase of hepcidin expression together with an increase of iron levels in the skin of the treated mice. In imiquimod treated mice deficient for hepcidin in keratinocytes, we found a significant reduction of epidermis thickness and a strong inhibition of neutrophils recruitment. Furthermore, a mouse model with specific overexpression of hepcidin in keratinocytes was sufficient to mimic these psoriasis features, emphasizing a causal role of hepcidin in psoriasis pathogenesis. In conclusion, our results suggest that hepcidin production in the skin could contribute to psoriasis development promoting 1-excessive iron accumulation leading to hyperproliferation of the epidermal layer and 2-recruitment of neutrophils leading to exacerbation of inflammation. Hepcidin antagonists and iron chelators constitute therefore a promising therapeutic target for psoriatic patients

O17

SKIN SENSORY DYSFUNCTION PRODUCED BY CHEMOTHERAPEUTIC DRUGS

Antonio Ferrer Montiel

Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universitas Miguel Hernández, Elche, Spain.

Chemotherapy-induced peripheral neuropathy (CIPN) is a severe adverse effect and highly prevalent disease occurring in up to 90% of the patients receiving most of chemotherapeutic drugs. CIPN sensory symptoms are characterized by paraesthesia, dysesthesia, spontaneous and burning pain, mechanical and thermal hypersensitivity primarily in the hands and feet. This sensory disorder resolves in 30% of patients after chemotherapy cessation but remains in up to 40% of patients for more than 1 year. Noteworthy, for some drug regimens ≈30% of patients must adjust or stop their cancer treatment because the intense sensory dysfunction produced by CIPN. Evidence from animal models suggests a drug-induced axonopathy of peripheral A- and C-type fibers that increases their excitability. The mechanisms underlying the alteration of the electrogenic activity of sensory neurons by chemotherapeutic drugs remain poorly understood. We have addressed this question using an *in vitro long term* pre-clinical nociceptor model that allowed us to investigate neural sensitization and its dissipation upon drug removal. We observed that 24h paclitaxel and 48h oxaliplatin exposure stimulated spontaneous activity and augmented repetitive action potential firing of nociceptors from male and female rats. Nociceptor sensitization was reversible peaking at 48h and dissipating at >96h post-drug exposure. Notably, paclitaxel augmented capsaicin and menthol currents, concomitant to an increase in the immunoreactivity of TRPV1 and TRPM8, while oxaliplatin incremented capsaicin and allyl-isothiocyanate (AITC) along with the expression of TRPV1 and TRPA1. Thus, these results signal to thermoTRP channels as therapeutic targets to reduce sensory symptoms in CIPN patients. Indeed, a topical formulation of a non-pungent capsaicin derivative notably reduced the disturbing sensory symptoms in hands and feet of a cohort of 27 cancer patients with CIPN grades I/II. Taken together, these findings indicate that skin sensory dysfunction in CIPN may be reduced by peripheral targeting of sensory receptors. (Funded by: PROMETEO/2021/031 (GVA); PID2021-126423OB-C21 (MICIN); PAR2019 (UMH); EU-956477 (MSCA-ETN PIANO, European Union Horizon 2020).

O18

DAYLIGHT PDT-THE SCOTTISH EXPERIENCE

M.O'Reilly, S.Ibbotson

Department of Dermatology, Ninewells Hospital, Dundee, Scotland

Daylight PDT (dPDT) is an effective, well-tolerated treatment for actinic keratoses (AK). We established a successful dPDT service in Scotland in 2013 and discovered that, despite our climate, this is a feasible therapy between April and September, with temperature rather than light being the limiting factor for successful treatment delivery. We report our experience of hospital-initiated daylight PDT in 186 patient treatment courses. The majority of patients (61%) achieved clearance or at least a good response and had high levels of satisfaction, reporting a preference for dPDT compared to other AK therapies (83%). We also established an innovative home-based dPDT service in 2021, enabling patients to self-treat in their home environment and results from this first year showed similarly high rates of efficacy (66%) and patient satisfaction. dPDT is an important treatment option for Scottish patients with AK and can be effectively delivered through both hospital-initiated and entirely home-based self-treatment approaches.

O19

MOLECULAR MECHANISMS OF MELANIN TRANSFER AND PROCESSING

Duarte Barral

NOVA Medical School – NOVA University of Lisbon, Lisbon, Portugal

Skin pigmentation relies on the pigment melanin, which protects skin cells against ultraviolet (UV) radiation-induced damage. Melanin is produced by melanocytes and then transferred to keratinocytes, where it is processed and forms supra-nuclear caps. Despite the crucial role of melanin secretion, transfer and processing by keratinocytes for skin pigmentation, the pathways involved remain controversial and poorly characterized. We have been studying these processes by characterizing the pathways and regulators involved. We found that basal melanin secretion is regulated by Rab11b and the exocyst complex; that melanin uptake occurs through PAR-2, Rac1- and Cdc42-dependent phagocytosis; and that melanin polarization within keratinocytes is dependent on Rab7-mediated transport along microtubules. Furthermore, we uncovered a new melanin exocytosis pathway, stimulated by keratinocyte-conditioned medium and regulated by Rab3a. These studies provide a better understanding of the skin pigmentary system and shed light on how skin phototype is determined, and how basal and facultative pigmentation are regulated.

O20

USING ZEBRAFISH TO STUDY THE MECHANISMS OF TISSUE REPAIR

David Gurevich[1,2], Charlie Severn[2], Catherine Twomey[2], Alex Greenhough[3], Jenna Cash[2], Ash Toye[2], Harry Mellor[2], Paul Martin[2].

Affiliation: 1) Department of Life Sciences, University of Bath, 2) Department of Biochemistry, University of Bristol, 3) Department of Cellular and Molecular Medicine, University of Bristol

Wounds that struggle to heal affect the quality of life for millions, incurring substantial costs to the NHS. Existing therapeutic interventions are largely ineffective, primarily due to a lack of understand of the mechanisms of tissue repair, and how these break down to result in compromised wound healing. Previous studies suggest this may be in large part due to poor control of inflammation and insufficient blood vessel supply to injured tissues. However, these studies could not directly live-image events as they occur. Our work demonstrated how zebrafish live-imaging in real time can be a powerful approach to dissect key interactions underlying repair. In particular, we investigated the cellular interactions between innate immune cells and endothelial cells at wounds, showing that macrophages are drawn to wound blood vessels soon after injury and are intimately associated throughout the repair process, and that macrophage ablation results in failure of vessel sprouting. Manipulation of the wound environment to alter macrophage activation state also dramatically influences subsequent blood vessel sprouting. Mechanistically, we have shown that pro-inflammatory macrophages associate with endothelial tip cells and are sufficient to drive vessel sprouting via Vascular Endothelial Growth Factor signalling. By establishing models of aberrant repair, such as foreign body implantation or diabetic wounds, we are using zebrafish to unravel how the interface between key repair processes is dysregulated in pathological contexts, which will ultimately provide valuable insight into novel approaches to rescuing tissue repair.

O21

STRETCHING THE SKIN TO FACILITATE NEEDLELESS DELIVERY OF VACCINES

Faiza Benaouda[1], Chui Hua Lim[1], Mohamed A. Alhnan[1], Mazen M. S. Aly[1], Daniel Sebastia-Saez[2], Rikhav P. Gala[3], Liang Cui[2], Tao Chen[2], and Stuart A. Jones[1].

[1] Institute of Pharmaceutical Science, Franklin-Wilkins Building. King's College London, Faculty of Life Sciences & Medicine, London. United Kingdom.[2]Department of Chemical and Process Engineering, University of Surrey, United Kingdom. [3] Fraunhofer USA Center for Molecular Biotechnology, United States.

The direct delivery of biological and gene-based therapeutics directly to the skin would allow immune targeting by direct access to the epidermis and the lymphatic system. However, the outermost layer of the skin, the *stratum corneum*, acts as an efficient barrier and does not permit direct administration of these agents. In this study, controlled skin stretching, induced using a patch system comprising a hypobaric chamber, was used as a non-invasive means to permeabilise the tissue and deliver an influenza vaccine antigen. Confocal imaging and finite element method (FEM) modelling indicated that controlled stretching of the skin induced a ~32% increase in the appendages diameter and led to a reduction in the skin thickness and compression of the epithelia lining the appendages. These changes were shown, through the skin permeation tracking of fluorescently labelled model macromolecules (FITC-dextrans with molecular weight of 4 kDa and 150 kDa), to modulate the transdermal delivery of the antigen. Once within the skin, analysis of the lymph nodes and whole body imaging demonstrated that the direct skin delivery facilitated lymphatic system targeting and this induced a superior antigen-specific IgG response (30.8 ± 8.7 mg/ml) ($p < 0.05$) compared to intramuscular injection (20.6 ± 1.7 mg/ml) (Fig.1).

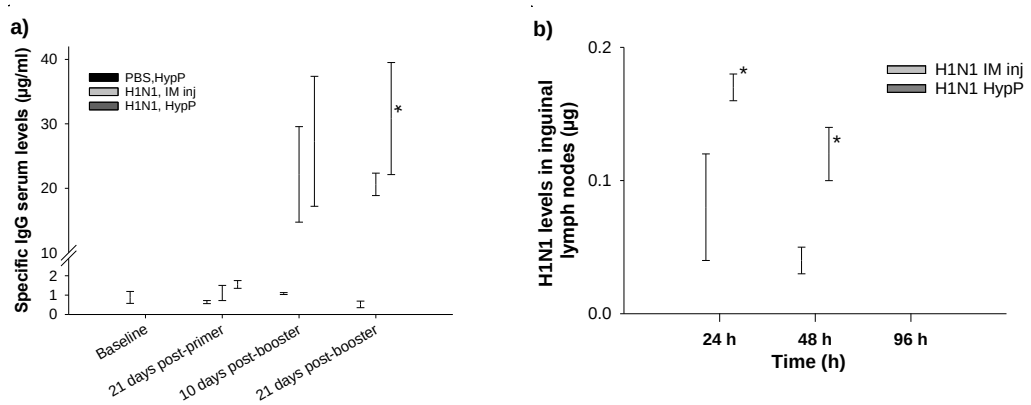


Fig.1. a) H1N1 HA Specific IgG response to the H1N1 antigen delivery into the skin using skin stretching under hypobaric pressure (HypP) and using intramuscular injection (IM inj). B) The amount of H1N1 antigen delivered into the inguinal lymph nodes at different time points after antigen delivery across the skin using stretching (HypP) and via intramuscular injection (IM inj) (n=6 ± SD).

This approach has the potential to facilitate the self-administration of therapeutics such as vaccines, RNA etc without the need of an injection system which could help the translation of these products into effective clinical use.

References

1) Anselmo, A. et al., Non-invasive delivery strategies for biologics. Nature Reviews Drug Discovery. 2019, 18, 19–40 (2019)

O22

Understanding vitamin D supplementation; effects of race and delivery route

Makiko Kawashita[1], Sumayah Abdul-jabbar[1], Charlie Lim[1], Chukwuebuka Nebechi[2], Graham R. McClelland[2], David Morgan[2], Guy Carpenter[3], Stuart A. Jones[1,2].

[1] Institute of Pharmaceutical Science, Franklin-Wilkins Building. King's College London (KCL), Faculty of Life Sciences & Medicine, London. United Kingdom. [2]Centre for Pharmaceutical Medicine Research, KCL. [3]Faculty of Dental, Oral & Craniofacial Sciences, KCL.

Vitamin D3 deficiency is a worldwide public health problem that can lead to a wide range of disorders including rickets, osteoporosis, and infections. Replacement therapy is typically administered orally at doses that are standardised to the level of perceived deficiency, but adherence to oral treatment is only 50-60% [1,2]. One solution to the supplement compliance problems is to administer vitamin D via the skin, but a novel approach is needed to deliver cholecalciferol, the molecule used in replacement therapy, transdermally, because it does not readily pass through the skin tissue. This project aimed to understand more about the response to oral replacement therapy in humans, identify the current replacement therapy needs, then design and test an innovative transdermal delivery approach. A systematic review of the clinical studies of oral vitamin D supplementation indicated that ethnic origin influenced the changes in 25(OH)D blood levels after oral vitamin D supplement administration (Figure 1). This suggested any transdermal approach should have dosing flexibility. In response to this need, a vitamin D phosphate-loaded adhesive patch was designed that could be produced in different sizes. In ex-vivo skin studies, 300 µg/cm^2 of vitamin D phosphate was delivered into the skin, but no active agent passed through the tissue. Metabolism studies demonstrated that 50% of the phosphate ester converted to vitamin D over 24 h. In a preliminary study in rats, the patch achieved higher blood metabolite levels compared to the oral tablet. Importantly, the expression of the vitamin D binding protein was induced in the rats, increasing from 300 µg/ml to almost 600 µg/ml after transdermal delivery, an effect that was not observed after oral administration.

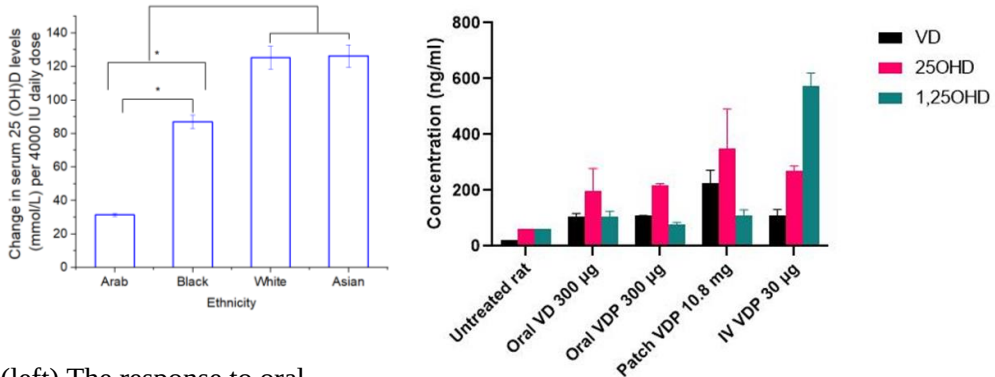


Figure 1. (left) The response to oral vitamin D supplementation across 19 clinical studies. (right) preliminary plasma vitamin D and vitamin D metabolite concentrations after different dosing routes (n=1 for the untreated and n=2 for the treated groups).

The transdermal patch will enter clinical testing in a four-arm double-blind placebo-controlled study in 2023 in order to assess its capability to become the first clinically effective vitamin D transdermal patch.

References: 1**.** Díez et al., Menopause. 2012 Jan;19(1):89-95; 2. Sanfelix-Genovés J, et al., Drugs Aging. 2009;26(10):861-9.

Poster Presentations

P1

TRANSCRIPTIONAL CO-REGULATOR YAP INTERACTS WITH RIF1 IN HUMAN CUTANEOUS SQUAMOUS CELL CARCINOMA

Jodie Bojko

Walko Laboratory, Department of Life sciences, University of Bath, BA2 7AY

Non-melanoma skin cancers (NMSC) are the most commonly diagnosed cancer worldwide with incidence increasing by 169% in the UK since the 1990s (cancer research UK). NMSC can be subdivided into two main categories: Basal Cell Carcinoma and Cutaneous squamous cell carcinoma (cSCC). YAP and TAZ are two paralogous transcriptional co-regulator proteins that are sequestered in the cytoplasm by the Hippo signalling pathway but nuclear when Hippo signalling is inactivated. YAP/TAZ are important in healthy skin for wound healing but also play a role in cSCC and function to influence gene transcription in favour of tumour growth by preventing cellular differentiation programmes. How YAP/TAZ implement such oncogenic transcriptional programmes is yet to be determined. Using RIME (Rapid Immunoprecipitation Mass Spectrometry) we have identified the nuclear YAP/TAZ interactome in a cSCC cell line. Of the proteins identified, Rif1 – a protein implicated in double-strand break repair and DNA replication timing – was selected for experimental validation using endogenous co-immunoprecipitation and proximity ligation assay (PLA) and was confirmed to be a protein interactor of YAP in parallel to YAP's gene regulatory functions. Knockdown of Rif1 and YAP in cSCC perturbed cell cycle progression, notably S-phase entry and progression. RNA sequencing following YAP/TAZ or Rif1 knockdown showed multiple differentially regulated genes and displayed that YAP/TAZ knockdown decreases the expression of RIF1. Using a stable shYAP cell line, Rif1 protein expression was shown to be decreased upon YAP knockdown suggesting YAP upregulates the expression of Rif1 in cSCC. Upon treatment with the replication stress-inducing chemotherapeutic, hydroxyurea, YAP-Rif1 PLA interactions increase following 24 hours of HU treatment, showing that YAP and Rif1 interact in response to replication stress and YAP protein levels are increased from as early as 30 minutes post-drug washout. This work shows YAP interacts with and upregulates the expression of Rif1 in cSCC and highlights unexplored functions of YAP outside of transcriptional regulation in cancer.

P2

HEPCIDIN, A NEW PLAYER IN PSORIASIS: ANALYSIS OF NEW MOUSE MODEL OVEREXPRESSING HEPCIDIN IN THE SKIN

Doha Chrayteh [1], Elise Abboud [1], Nadia Boussetta [1,] Héloïse Dalle [1], Sophie Vaulont [1], Joël Poupon [2], Pierre Sohier [1 3], Selim Aractingi [1 4], Zoubida Karim [5], Carole Peyssonnaux [1].

1Université Paris Cité, CNRS, INSERM, Institut Cochin, F-75014 Paris, France 2 Laboratoire de Toxicologie Biologique, Hôpital Lariboisière, Paris, France 3 Service de Pathologie, AP-HP, Hôpital Cochin, F-75014 Paris, France 4 Service de Dermatologie, AP-HP, Hôpital Cochin Tarnier, F-75006 Paris, France 5 Institut des Maladies Infectieuses et Inflammatoires de Toulouse, France.

Hepcidin, a hormone mainly produced by the liver is the central regulator of systemic iron homeostasis. We have recently shown that hepcidin can be produced by keratinocytes and play under infectious conditions, an immunomodulatory role in the skin by promoting the production of the chemokine CXCL1 by keratinocytes, and the consecutive neutrophil recruitment. To determine the role of hepcidin in the skin, our laboratory generated a new mouse model, using a cre-loxP knock-in approach (in collaboration with Dr. Zoubida Karim) in which hepcidin is specifically overexpressed in keratinocytes. Interestingly, the new Hepc1KI-Ker mouse model developed alopecia and up to 50% mortality rate. These mice presented with pronounced acanthosis, iron retention and increased epidermal layer proliferation. In addition, overexpression of epidermal hepcidin induced an upregulation of CXCL1 and neutrophil recruitment.

Besides, epidermal hepcidin overexpression resulted in a systemic iron deficiency phenotype associated with low hepcidin level in the liver, which may result from increased iron loss through hyperproliferation and desquamation of the epidermal layer of the skin. These results suggest a potential role of keratinocyte-derived hepcidin in cutaneous inflammatory skin diseases such as psoriasis, characterized by skin iron retention, acanthosis and neutrophil recruitment as well as a role of skin hepcidin in systemic iron homeostasis.

P3

INVESTIGATING THE THERAPEUTIC EFFECTS OF DIFFERENT ANTIOXIDANTS IN FRIEDREICH'S ATAXIA

Fred Jonathan Edzeamey[1], Adamo Valle Gómez[2], Charareh Pourzand[3], Robert Hider[4], Ronan McCarthy[1], Sara Anjomani Virmouni[1]

[1]*Division of Biosciences, College of Health, Medicine, and Life Sciences (CHMLS), Institute of Environment, Health & Societies, Brunel University London, UK.*

[2]*Energy Metabolism and Nutrition, University of Balearic Islands, Research Institute of Health Sciences (IUNICS) and Medical Research Institute of Palma (IdISPa), Spain.*

[3]*Department of Pharmacy and Pharmacology, University of Bath, UK.*

[4]*King's College London, UK*

Aberrant frataxin expression in Friedreich's ataxia (FRDA) results in increased oxidative stress and mitochondrial dysfunction, driving disease progression. Different antioxidants have been tested in the past with quite satisfying and encouraging results *in vitro* and *in vivo*. So far there is no cure or effective treatment for FRDA. This project aims at investigating the effect of novel antioxidants on mitochondrial redox homeostasis and metabolism in FRDA.

Candidate compounds selected include Dimethylfumarate, N-acetylcysteine, Resveratrol, Vitamin C and E and Lipoic acid. *Galleria mellonella* model was used to determine the LD_{50} of potential antioxidants. Toxicity of compounds in FRDA cells was assessed using PrestoBlue cell viability assay. Mitochondrial ROS (mROS)-decreasing capacity of compounds in FRDA fibroblast cells was assessed by MitoSOX-based flow cytometric assay, and *FXN* and NRF2 gene expression levels were assessed using qRT-PCR. The cytoprotective effects of compounds against H_2O_2-induced toxicity and the effects of compounds on cellular ROS and mitochondrial membrane potential were also assessed using their respective probes by flow cytometry. Toxicity of the antioxidants was first assessed in the *Galleria* model and those compounds demonstrating minimal toxicity were further tested *in vitro* FRDA cell models. Cell viability studies in FRDA fibroblasts revealed 15μM Resveratrol, 2mM Vitamin E, 440μM Vitamin C, 0.1μM Lipoic acid, 10μM Dimethylfumarate and 1.5mM N-acetylcysteine as the tolerable concentrations. Significant reduction in mROS and cellular ROS were observed in FRDA cells following treatment with Vitamin C, N-acetylcysteine, Resveratrol, Dimethylfumarate, and Vitamin E. These compounds were also found to significantly increase the levels of *FXN* and NRF2 gene expression, and to protect FRDA cells from H_2O_2-induced toxicity and further mitigate the collapse of mitochondrial membrane potential. The effect of these compounds on frataxin, Nrf2, catalase and glutathione peroxidase protein expression and other mitochondrial enzymes are currently being assessed.

The efficacy and safety of the antioxidants have been tested in FRDA fibroblasts. The ability of the compounds to upregulate the antioxidant response elements will be further assessed. Candidate compounds will be modified to improve their efficacy/bioavailability and facilitate their clinical translation. This study may provide a novel therapeutic avenue for FRDA.

P4

UNDERSTANDING THE THERAPEUTIC POTENTIAL OF HYDROGEN SULFIDE FOR TREATING MICROBIAL SKIN INFECTIONS

Fritz Ka-Ho Ho[1], Sara Nasereddin[2], Stuart Jones[2], Albert Bolhuis[1]

[1] Department of Life Science, University of Bath, UK
[2] Institute of Pharmaceutical Science, King's College London. UK

Background

Skin and nail infections with fungi and bacteria are very common, affecting 20-25% of the world population. The administration of antimicrobials is challenging due to the properties of skin and nails as protective tissues of the body. Topical treatments can be lengthy and often fail due to poor penetration of antimicrobials. However, we hypothesise that hydrogen sulfide (H_2S), which is small and has antimicrobial activity, provides an alternative treatment strategy as this may penetrate much better into skin or nails as compared to conventional antimicrobial agents. This study is to understand the effectiveness and mechanism of actions of H_2S to pathogens that cause skin and nail infections, including fungal dermatophytes and *Staphylococcus aureus*.

Methods

The H_2S was generated by the H_2S donor NaHS. To identify the antifungal and antibacterial activities, fungicidal and serial dilution spot assays were conducted. The response of reactive oxygen species (ROS) was visualised by DCHF-DA through confocal microscopy using a redox-sensitive dye. The effect of H_2S on the activity of cytochrome *c* oxidase (COX) was monitored by a COX activity assay on isolated mitochondria from the dermatophyte *Trichophyton rubrum*.

Results

H_2S was shown to be fungicidal against *T. rubrum* and *Trichophyton interdigitale*. H_2S was fungistatic against other fungi that cause nail infections, including *Aspergillus niger*, *Neoscytalidium dimidiatum*, *Fusarium oxysporum*, and *Candida albicans*. Early confocal and flow cytometry studies showed that H_2S kills dermatophytes in a time- and fungal life cycle-dependent manner. It also has a strong bactericidal activity against methicillin-resistant *S. aureus* (MRSA), even at high bacterial density levels. The mechanism of action is not clear yet, but early results suggest that H_2S leads to the production of ROS and inhibition of the COX complex.

Conclusion

The study demonstrated the antimicrobial activity of H_2S produced by NaHS on several dermatophytes and MRSA.

to reformulate these supplements as transdermal patches.

P5

Investigating the roles of YAP and TAZ in human cSCC progression - Abstract – Skin@Bath 2022

Alexander Howard –

Walko Lab, University of Bath

Introduction

Non-melanoma skin cancers (NMSCs) are amongst the most frequently diagnosed human cancers. Cutaneous squamous cell carcinoma (cSCC) accounts for the majority of deaths associated with NMSCs. Like other cancers, cSCC arises from genetic alterations that invariably lead to dysregulated transcriptional programmes. Studies in mice demonstrated an absolute requirement of the transcriptional co-regulator proteins YAP and TAZ (WWTR1) for cSCC initiation and progression in murine epidermis. The majority of past studies on YAP/TAZ focused on either one of the paralogues or had assumed similar functions between them. However, emerging evidence demonstrates that YAP and TAZ have distinct roles where they partner with different transcription factors, to drive different transcriptional programmes. We have therefore assessed distinct and non-redundant roles of YAP and TAZ in a human cSCC cell culture model.

Materials and methods

Our human cSCC model consists of a panel of isogenic human epidermal cell lines representing different stages of neoplastic progression (MET1, MET2, MET4), which were derived from the clinical progression of a primary human cSCC through to distant metastasis in a single immunosuppressed individual. We generated sublines of MET1, MET2 and MET4 expressing YAP-/TAZ-targeting inducible shRNAs to study the functions of YAP and TAZ using variety of 2D and 3D cell culture assays, immunofluorescence microscopy, western blotting, and published microarray data for bioinformatics analysis.

Results and discussion

We observed variable requirement of YAP and TAZ for cSCC proliferation in 2D and 3D culture conditions: MET1 (cSCC in-situ) was strongly dependent on YAP, MET2 (invasive cSCC, poorly differentiated recurrence of MET1) was dependent on both YAP and TAZ, whereas MET4 (lymph node metastasis of MET1/MET2) was not dependent on either paralogue. Surprisingly, we observed no obvious correlation between sensitivity to YAP/TAZ depletion and YAP/TAZ expression itself. In addition, YAP/TAZ nuclear/cytoplasmic ratio (often used as a proxy for YAP/TAZ activity) was highest in MET4 cells, despite their independence on YAP/TAZ for cell proliferation.

Conclusion

Our findings suggest that YAP is the pre-dominant paralogue in cSCC but appears to cooperate with TAZ during cSCC invasion. However, once cSCC cells have metastasised, they appear to no longer depend on YAP/TAZ to thrive. Altogether, our findings strongly argue against the use of YAP/TAZ expression and YAP/TAZ nuclear/cytoplasmic ratio as predictors for YAP/TAZ activity in cSCC. As analysis of YAP/TAZ target gene expression provides a more robust and quantifiable way to assess YAP/TAZ activity than measuring its expression level, subcellular localisation or phosphorylation, our future work will aim to identify a cSCC-specific YAP/TAZ transcriptional signature.

P6

INVESTIGATING FUNCTIONS FOR SYNTENIC LONG NON-CODING RNAS DURING ZEBRAFISH MELANOCYTE DEVELOPMENT AND HUMAN MELANOMA

Stephanie Jones, Keith Vance, Robert Kelsh

University of Bath, Department of Life Sciences.

Melanoma is an aggressive skin cancer which derives from the malignant transformation of melanocytes. Melanoma is associated with a poor prognosis once metastasised, with switching phenotypes to a de-differentiated neural crest stem cell-like state hypothesised to contribute to therapeutic resistance. During development, neural crest cells give rise to several types of cells including melanocytes. Thus, highlighting the need for further understanding of the molecular mechanisms which govern the progression of melanoma as well as their associated developmental programmes. Long non-coding RNAs (lncRNAs) have recently been shown to play a role in tumorigenesis and progression of melanoma. LncRNAs are a class of genes that are not translated into proteins but have been found to be dysregulated in cancer. Thousands of lncRNAs have been catalogued however, our understanding of their mechanisms which govern melanoma progression and neural crest development remains limited.

The aim of this study is to identify and characterise the function of positionally equivalent lncRNAs regulated by MITF-SOX10. It is hypothesised that these lncRNAs will be imperative for neural crest cell differentiation, melanocyte development and melanoma progression. A total of 24 transcripts were identified between human and zebrafish genomes whose loci are predicted to be bound by MITF-SOX10. *LINC00327* was chosen for further study and MITF-SOX10 regulation confirmed. We discovered conserved regulation of *Linc00327* by *Sox10* in both human melanoma and zebrafish neural crest cells, a novel finding which to our knowledge has not been shown in the lncRNA field before. In human melanoma, *LINC00327* was shown to suppress both proliferation and migration, two key cancer hallmarks. We now hypothesise that *LINC00327* is a novel melanoma tumour suppressor which we predict to have an important role in neural crest development.

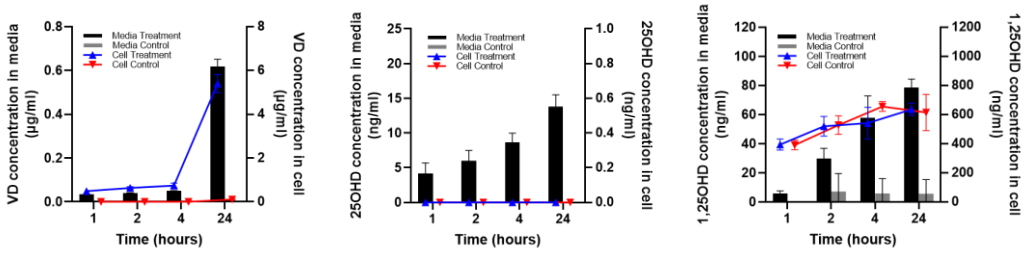
P7

UNDERSTANDING SKIN METABOLISM OF TRANSDERMALLY DELIVERED PHOSPHORYLATED VITAMIN D_3

C. Lim [1], P. Luebcke [2], M. Alhnan [1], S. A. Jones [1]

[1]*Institute of Pharmaceutical Science, King's College London, Faculty of Life Science & Medicine, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom*
[2]*Curapel Ltd, Stuart House, Chepstow NP16 5HH United Kingdom.*

Vitamin D (VD) deficiency remains a global problem despite the availability of oral supplementation. Skin generated VD is more biological active compared to oral VD, thus transdermal delivery of VD, which represents an alternative source of VD from the skin, has the potential to improve VD status more efficiently compared to oral route. However, VD cannot pass through the skin efficiently. A new phosphate analogue of VD has overcome this issue. The aim of this study is to gain ground understanding of VD metabolism in the skin when delivered via transdermal route, as well as the extent and rate of conversion of VDP to VD using in vitro cell culture model. Cell viability of rat epidermal keratinocytes (REK) on VD and VDP doses was determined using MTT assay. Metabolism study was conducted by incubating REKs with VD and VDP doses over 24 h. Extracellular and intracellular VD metabolites levels were analysed with GC-MS method. Results showed conversion of VDP to VD within one hour of dose treatment, achieving 50% dose conversion at 24 h. This could be attributed to the structural similarity of VDP to VD esters naturally formed in the skin, which are hydrolysed into active VD before entering systemic circulation. Conversion of VDP to VD was found to be dose dependent, with 10 µM and 100 µM VDP producing 1.6 µM and 9.3 µM VD respectively at 24 h. 1% of VD and 2 % of VDP administered was metabolised by the keratinocytes into the active metabolite, calcitriol ($1,25OH_2D$). This may contribute to improved bioactivity of transdermal VD compared to oral route which has to rely on renal metabolism for calcitriol production.



Metabolism of VDP into VD and active metabolites over 24 h.

This work suggested that phosphorylated VD could be highly effective for transdermal delivery as it is converted spontaneously into VD in the skin.

References

1) Duchow, E. G., *et al. Physiological reports*, **9**(23), e15138 (2021).
2) Duchow, E. G., *et al. Photochemical & photobiological sciences*, **21**(8), 1399–1404 (2022).

P8

UNDERSTANDING BARRIER PROPERTIES OF THE KERATIN-RICH TISSUES

Sara Nasereddin[1], Osamah Malallah[1], Albert Bolhuis [2],Mohammad Alhnan[1] , Stuart A. Jones[1]

[1]*Institute of Pharmaceutical Sciences, Faculty of Life Sciences & Medicine, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NH, UK*
[2] *Department of Pharmacy and Pharmacology, University of Bath, Calverton Down, Bath BA2 7AY, UK*

Onychomycosis is a fungal infection of the nail that accounts for approximately 50% of all nail diseases worldwide (1). Current treatment for this nail disease lack efficacy due to the poor penetration through the nail plate and a narrow antimicrobial spectrum of action (2,3). The human nail is composed of hard and soft keratin (4). These keratins come together to form the functional region of the nail, known as the cortex (4). The cortex consists of two elements, the inner element known as the intermediate filaments (IFs), and the outer, protective element known as the intermediate filaments associated proteins (IFAPs), bound together by disulphide bonds (5). However, it remains unclear which element is responsible for the barrier properties of these keratin-rich tissues. The aim was to understand the properties of keratin barriers to enable the subsequent engineering of novel molecules with high permeability to treat local nail infections. Keratin tissues studied were clippings of the human nail, rhino horn and porcine hoof soaked with reducing agent solutions thioglycolic acid, tris (2-carboxyethyl)-phosphine and phosphate buffer saline as a control for 24 h at 30°C. The clippings were then analysed using Raman spectroscopy (disulphide bond breakage), ATF-FTIR (Amide I protein denaturation), water up test (swelling), and Franz diffusion cells (structural integrity) using Rhodamine B and Lucifer Yellow. All disulphide bond-reducing agents showed the same effect in breaking the disulphide bond in the three tissues, but the peaks had different wavenumbers. Tris (2-carboxyethyl)-phosphine showed a denaturating effect on the keratin turn in the nail and horn, but not the trotter. Thioglycolic acid had a greater effect on the water uptake in the nail and horn than in the trotter. The nail had a higher affinity for hydrophilic molecules than lipophilic molecules. Thioglycolic acid seemed to enhance the permeability through the opening of the hydrophilic diffusion pathway, IFAPs, whereas Tris (2-carboxyethyl)-phosphine enhanced permeability through the denaturation of IFs. This suggests that the disulphide bonds in keratin-rich tissues are responsible for the barrier properties. The type and location of these in the different types of tissues provide very different tissue permeabilization effects when they are broken.

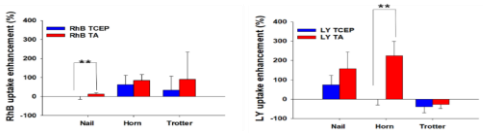


Figure 1. The effect of TA and TCEP on the deposition

References

(1) *Gupta AK, Mays RR. The impact of onychomycosis on quality of life: a systematic review of the available literature. Skin Appendage Disorders. 2018;4(4):208-16.*

(2) *Rajendra, V. B. et al. (2012) 'Transungual drug delivery: An overview, Journal of Applied Pharmaceutical Science.*

(3) *Aggarwal R, Targhotra M, Kumar B, Sahoo PK, Chauhan MK. Treatment and management strategies of onychomycosis. J Mycol Med. 2020;30(2):100949. doi: 10.1016/j.mycmed.2020.100949.*

(4) *Gniadecka, M. et al. (1998) 'Structure of water, proteins, and lipids in intact human skin, hair, and nail', Journal of Investigative Dermatology. Elsevier Masson SAS, 110(4), pp. 393–398. doi: 10.1046/j.1523-1747.1998.00146.x.*

(5) *Baswan, S. et al. (2017) 'Understanding the formidable nail barrier: A review of the nail microstructure, composition and diseases', Mycoses. DOI: 10.1111/myc.12592.*

P9

THE ROLE OF ACSL4 AND LPCAT2 IN RSL-3-INDUCED MITOCHONDRIAL PATHWAYS OF FERROPTOSIS IN HEK293T CELLS

Melanie Merkel[1,2], Bjarne Göbel[3], Dieter Steinhilber[3], Carsten Culmsee[1,2]

[1]Institute for Pharmacology and Clinical Pharmacy, Biochemical-Pharmacological Center, University of Marburg, Marburg, Germany; [2]Center of Mind, Brain and Behavior – CMBB, University of Marburg, Marburg, Germany; [3]Institute of Pharmaceutical Chemistry, University of Frankfurt, Frankfurt, Germany

Ferroptosis is a form of oxidative regulated cell death that is induced by enhanced lipid peroxidation upon disruption of lipid repair systems engaging glutathione and glutathione peroxidase (GPx4). Biosynthesis of polyunsaturated fatty acid (PUFA)-containing phospholipids is a prerequisite for initiation of ferroptosis [1], and ferroptosis is accelerated in cells with high levels of plasma membrane phosphatidylethanolamines (PE) [2]. The Acyl-CoA synthetase long-chain family member 4 (ACSL4) drives esterification of arachidonic acid (AA) and adrenic acid (AdA) into PE, thereby promoting ferroptosis. In addition, the re-acylation of the major membrane phospholipid, phosphatidylcholine, is mediated by a family of lysophosphatidylcholine acyltransferase (LPCAT) enzymes, thereby facilitating lipid peroxidation [3]. Whether ferroptosis initiated through ACSL4/LPCAT2 requires mitochondrial mechanisms of ferroptosis [4] has not been clarified. Therefore, we investigated whether overexpression of ACSL4 and LPCAT2 affected mitochondrial mechanisms of oxidative cell death in a model of RSL3-mediated ferroptosis and evaluated strategies of mitochondria-targeted protection.To examine the impact of the two enzymes in ferroptosis, Hek293T cells were stably transfected by the Sleeping Beauty System [5] for overexpression of ACSL4 and LPCAT2 (OE) and empty vector control cells (LV). The quantification of protein and mRNA levels of ACSL4/LPCAT2 was achieved by Western blot and qPCR. Both cell lines were treated with RSL-3 to induce oxidative cell death and metabolic activity (MTT assay) was measured afterward to determine cell viability. Flow cytometric (FACS) measurements were conducted for detecting cell death (Propidium iodide), mitochondrial membrane potential (TMRE), and mitochondrial reactive oxygen species (ROS) formation (MitoSOX). Further, Seahorse XF Analyzer was used for measuring mitochondrial respiration. ACSL4/LPCAT2 overexpressing cells resulted in a higher sensitivity against RSL-3-induced ferroptosis compared to LV-transfected controls, as demonstrated by a concentration-dependent reduction of the cell viability. This detrimental effect was also confirmed by FACS analysis of PI uptake, decrease of mitochondrial membrane potential, and increased ROS production after RSL-3 treatment. Nevertheless, these effects were reversed by Ferrostatin-1 and Deferoxamine. To further investigate the role of mitochondria, the mitochondrial ROS scavenger Mitoquinone (MitoQ) was applied, resulting in full protection from RSL-mediated mitochondrial damage as detected in the TMRE and MitoSOX assays. Moreover, pharmacological ACSL4 inhibition by Troglitazone or Rosiglitazone prevented oxidative cell death in both cell lines.

These results showed that the overexpression of ACSL4 and LPCAT2 accelerated ferroptosis in Hek293T cells in comparison to LV cells. Inhibiting ACSL4 reduced cell death and preserved mitochondrial integrity and function. Further, the mitochondrial-targeted radical scavenger MitoQ resulted in reduced mitochondrial ROS production, and preserved mitochondrial membrane potential, implicating that mitochondria are key mediators of oxidative cell death initiated through lipid peroxidation in ferroptosis.

1. Stockwell, et al.: Cell. 2017, 171: 273-285.; 2. Kagan, et al.: Nat Chem Biol. 2017, 13: 81–90.3. Mishima and Conrad.: Annu. Rev. Nutr. 2022. 42: 15.1–1

4. Neitemeier et al., Redox Biol. 2017,

5. Kowarz, et al.: Biotechnol. J. 2015, 10: 647–653.

Acknowledgements: This work received financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e Tecnologia), under the Partnership Agreement PT2020 through project UID/QUI/50006/2013-POCI/01/0145/FEDER/007265 (LAQV/REQUIMTE).

P10

INTERACTION OF SKIN CELLS WITH SKIN MICROBIOTA

MOINIPOOR Zeynab, MURPHY John, ZARIWALA Mohammed Gulrez, KESHAVARZ Tajalli,

University of Westminster – London – United Kingdom

Introduction: The interaction between skin cells and skin microbiota is complicated and not fully understood. Millions of microbial cells living on human skin suggest the importance of studying the effect of skin microbiota and their contribution to skin health and disease. Several studies specially on gut microbiota, have correlated the microbiota composition with gut health and disease. *Pseudomonas aeruginosa* is one of the leading opportunistic pathogens in chronic wound infections. Biofilm formation of *P. aeruginosa* in the wound area following its attachment to the cells has a deleterious impact on wound healing. On the other hand, *Staphylococcus epidermidis*, as a skin commensal, has been shown to protect the skin against pathogens. Therefore, this study is aimed at investigating the effect of skin commensal bacteria on the skin cells and opportunistic bacteria.

Materials and Methods: The effect of *S. epidermidis* NCTC11047 supernatant on the biofilm formation, virulence factors production, and quorum sensing network of *P. aeruginosa* NCTC10662 and PAO1 was investigated using a range of colorimetric assays and RT-qPCR. Also, the effect of *S. epidermidis* supernatant on the viability, migration, and proliferation of the human skin keratinocytes (HaCaT cell line) was investigated using scratch and MTT assays.

Results: *S. epidermidis* supernatant diminished biofilm formation and virulence factor production of *P. aeruginosa* without any impact on growth through modulation of quorum sensing network. *S. epidermidis* supernatant also improved the proliferation and wound healing in keratinocytes.

Conclusion: With the increase in the prevalence of multi-drug resistant pathogens, discovery and application of molecules offering anti-virulence properties may be a promising alternative therapy in infected wounds.

References:

Byrd, A.L., Belkaid, Y., Segre, J.A. (2018). The human skin microbiome. Nature Reviews Microbiology. 16 (3), 143-155.

Brown, J.L., Townsend, E., Short, R.D., Williams, C., Woodall, C., Nile, C.J., Ramage, G. (2022). Assessing the inflammatory response to in vitro polymicrobial wound biofilms in a skin epidermis model. NPJ Biofilms and Microbiome.

P11

RAPID™ L-PRP GEL EXUDATE CONTAINS GROWTH FACTORS AND PROMOTES THE PROLIFERATION OF IMMORTALIZED HUMAN KERATINOCYTES, HACAT CELLS

Aleksandra Olszewska[a], Duan JiaJing[a], Simon Pitchford [a], James Rickard [b] and Ben Forbes[a]
[a]King's College London, Institute of Pharmaceutical Science, 150 Stamford Street, SE1 9NH, London
[b]Biotherapy Services Ltd, Gainsborough House, 59-60 Thames Street, Windsor, SL4 1TX

RAPID™ leukocyte and platelet-rich plasma (L-PRP) gel is a novel therapeutic currently in stage 2b of clinical trials for diabetic foot ulcers (DFU), having been observed to shorted time-to-heal, accelerating wound closure and resolution of DFU. It is not clear what factors are important to the therapeutic success of the RAPID™. This study investigated the selected growth factor concentrations in the RAPID™ gel releasate (exudate) over time together with the effects of exudate and L-PRP - an unactivated component of RAPID™ - on the proliferation of human keratinocytes (HaCat cells) *in vitro*. The gels were incubated in a humidified incubator; at selected time points exudate was collected, and all samples frozen at -80℃ prior to analysis by Sandwich ELISA to quantify PDGF and VEGF. HaCat cells were seeded in a 96-well plate (2000 cells/well) and after a 24-hour cell attachment period, various concentrations of L-PRP and Exudate were added to the keratinocytes and incubated for 24 hours. The Resazurin assay was used to quantify cell proliferation over a 72-hour period. The results showed variability between gels, which was attributed to blood donor variability (platelet counts $190 - 380 \times 10^6$ platelets/ml, n=3). Proliferation was significantly greater at the 24 hour timepoint $(0.14x)$ between exudate and L-PRP concentrations 0.2, 1.0 and 2.0 % v/v. VEGF concentration in exudate was 311.8 ± 105.5 pg/mL and 640.0 ± 171.9 pg/mL at 1 and 3 hours, respectively. PDGF concentration in exudate was 1388.7 ± 824.6 pg/mL and 1926.7 ± 1125.9 pg/mL at 1 and 4 hours. These results show high contents of key growth factors – VEGF and PDGF; these may play a role in the proliferative effects of the Exudate. Further experimentation is needed to quantify other proteins that may play a role in the therapeutic success of RAPID™ gels.

P12

TARGETING IRON IN PSORIATIC SKIN, A NOVEL APPROACH FOR TOPICAL THERAPY OF PLAQUE PSORIASIS

Rengganis Ayu Pramudya Wardhani[1], Zihan Zhou[2], Agostino Cilibrizzi[3,4], Julia Li Zhong[1,5], Robert Hider[3], Charareh Pourzand[2,4]

[1]College of Bioengineering, "111 Project" Laboratory of Biomechanics & Tissue Repair Engineering, Key Laboratory of Biorheological Science and Technology, Ministry of Education, Chongqing University, Chongqing, China.[2]Department of Life Sciences, University of Bath, Bath, UK. [3]Institute of Pharmaceutical Science, King's College London, London, UK. [4]Centre for Therapeutic Innovation, University of Bath, Bath, UK. [5]Department of Dermatology, Chongqing Traditional Chinese Medicine Hospital, Chongqing, China

Psoriasis is a chronic, immune-mediated skin disease characterized by keratinocyte hyperproliferation and epidermal acanthosis (hyperplasia) and accompanied by increased infiltration of immune cells. Iron exerts a crucial role in cell proliferation and psoriatic keratinocytes have an increased need in iron, due to their high proliferation rate, which makes them particularly sensitive to changes in iron availability in the environment, in comparison to healthy keratinocytes. This difference can be exploited for anti-psoriatic therapeutic purposes through the use of iron chelators as potent antiproliferative agents. This study was designed to evaluate the antiproliferative and anti-inflammatory effects of two members of the 3-hydroxypyridin-4-ones (HPOs) iron chelator family (HPO1 and HPO2) for topical therapy of plaque psoriasis in both cultured skin cells (HaCaT keratinocytes) and an *in vivo* imiquimod-based psoriasis mice model. Using MTT, flow-cytometry based bromodeoxuuridine and cell doubling counting assays, we demonstrated that both HPO-1 and HPO-2 exhibit potent anti-proliferative activities in HaCaT cells that are comparable to that observed with clinical iron chelators Desferrioxamine and Deferiprone, respectively. By making the use of Imiquimod(IMQ)-induced psoriasiform dermatitis, we have further established that compared to HPO-2, HPO-1 topical application for 5 consecutive days had a strong beneficial effect on psoriasis-like skin lesion symptoms. Accordingly, the dorsal skin of IMQ+HPO1-treated mice, exhibited lower inflammatory cytokine expressions including TNF α, IL-6, and IL-17A than IMQ+HPO2-treated mice, when compared to IMQ-treated mice alone. Taken together, these data strongly suggest that HPO1 may be a suitable drug for the topical therapy of plaque psoriasis and possibly scalp psoriasis.

P13

ADDITIVE EFFECT OF HYDROGEN SULFIDE AND AMPHOTERICIN IN TREATING FUNGAL BIOFILMS

Costanza Pregnolato[1,2], Barry Panaretou[2], Tony Lockett[1,2], Alex Dyson[1,2]

[1]Centre for Pharmaceutical Medicine Research, Kings College London, London, UK
[2]Institute of Pharmaceutical Science, Kings College London, London, UK

Introduction: Biofilms are present in 90% of chronic wounds. They hinder wound healing and their removal is an ongoing challenge due to their strong adherent properties, ability to evade the local immune response, and resistance to traditional antimicrobial treatment [1]. Amphotericin B is an antifungal agent that has severe side effects when administered systemically e.g. nephrotoxicity; its topical application is emerging in case studies [2]. We investigated its utility in combination with hydrogen sulfide (H_2S), a small gaseous signalling molecule, in preventing formation and enabling degradation in biofilms of *C. albicans*.
Methods: An *in vitro* 96-well plate assay was used to assess biofilm formation and degradation [3]. *C. albicans* (SC5314; $1x10^6$ cells/mL) were plated in each well with biofilm formation assessed by absorbance spectrophotometry (λ: 600 nm). Prevention of biofilm formation by amphotericin (1 ug/ml) and sodium hydrogen sulfide (NaHS; 10 μM), alone or in combination, was assessed following addition of these drugs to freshly plated planktonic cells, and incubated for 24 hours at 37°C. In separate experiments, drugs were added to established (24h) biofilms, with a further 24h incubation (37°C) and subsequent measurement. **Results**: Amphotericin was effective at preventing biofilm formation with or without addition of NaHS (Fig 1A) but had no significant impact on established biofilms (Fig 1B). While NaHS (at 10 μM) alone could not prevent formation or degrade established biofilms, it enabled a significant ($p<0.05$) additive effect in combination with amphotericin (Fig 1B).

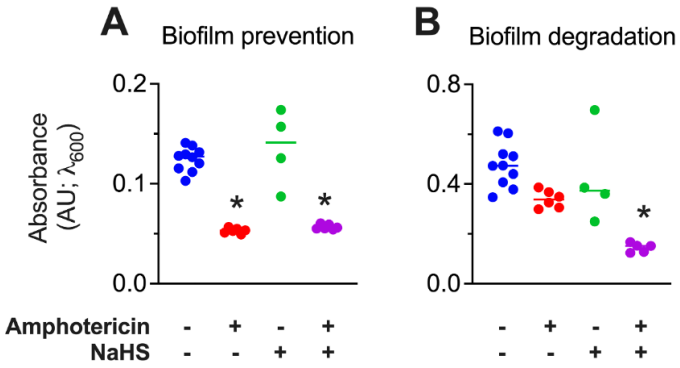


*Figure 1: Impact of amphotericin and NaHS in preventing biofilm formation (A) and enabling degradation (B). Data shown as individual values and median. *p<0.05 vs control; Kruskal-Wallis + Dunn's test, n=4-10/group. Prism 9 (GraphPad) was used for all analyses.*

Conclusion: The combination of H_2S and a traditional antifungal agent showed an additive effect in degrading fungal biofilms. This outlines a potential clinically utility for H_2S as an adjunct antifungal agent that could enable greater efficacy and spare the use of traditional antifungal agents.

References:
[1] Attinger C & Wolcott R. *Adv. Wound Care* 2012; 1:127-132.
[2] Melgarejo-Ortuño *et al. Am. J. Ther.* 2020; 27:e670-e671
[3] Pierce CG *et al. Nat. Prot.* 2012; 3:1494-1500.

P14

APPPLICATION OF WOOD EAR MUSHROOM FOR SKIN WOUND HEALING: THE INVESTIGATION OF WOUND HEALING PROPERTIES USING IN VITRO AND IN VIVO MODELS

Warathit Semmarath[1,2,3], Sonthaya Umsumarng[1,2,4], Sariya Mapoung[1,2], Kamonwan Srisawad[1,2], Punnida Arjsri[1,2], and Pornngarm Dejkriengkraikul[1,2]*

[1] Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand ; [2] Center for Research and Development of Natural Products for Health, Chiang Mai University, Chiang Mai 50200, Thailand ; [3] Akkraratchkumari Veterinary College, Walailak University, Nakhon Si Thammarat 80160, Thailand; [4] Division of Veterinary Preclinical Sciences, Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand

The wound-healing process is a multifaceted series of cellular and molecular events with the involvement of skin keratinocytes and fibroblasts to restore the integrity of wounded skin. Although the wound healing process can simultaneously occur, many factors can interfere with the wound healing and resulting in a delay of wound repairing. In recent years, researchers have investigated the wound healing properties of polysaccharide from plants and mushrooms. In our study, we proposed the wood ear mushroom, Auricularia auricula-judae, as a potential wound healing agent by using the in vitro and in vivo model to determine its wound healing properties. The polysaccharide-rich extract from wood ear mushroom (PWME) was established using hot water extraction and 95% ethanol precipitation. Using gel filtration and GC-MS analysis, we found that polysaccharide from PWME is characterized by its 158 kDa of molecular weight with mannose: galactose: glucose monosaccharide composition in a molar ratio of 1.49:0.21:0.10, respectively. PWME possessed an in vitro wound-healing activity in keratinocytes (HaCat) and primary dermal fibroblasts through the significant induction of cells proliferation and facilitating both migration and invasion processes. The effect of PWME on cell migration was confirmed by the decreased expression of the E-cadherin of keratinocytes using western blot. Additionally, PWME enhanced the collagen synthesis in dermal fibroblasts. We further confirmed the wound healing properties of PWME using the in vivo skin wound healing in BALB/C mice model. We found that 2.5% w/v PWME-treated mice (n=7) displayed an acceleration of wound closure in full skin thickness excision over the 12-day duration when compared with vehicle control group (0.5% normal saline). Microscopically, PWME-treated groups showed the thickening of epidermis and reduction of granulation tissue at the 12-day after full skin thickness excision. Additionally, the dermal layer showed the accumulation of fibroblast cells (H&E staining), together with dense collagen network (Masson's Trichrome) more prominent than the vehicle control group. Our data displayed the in vitro and in vivo wound healing properties of polysaccharides-rich extract from wood ear mushroom which can be applied as a potential candidate for wound-healing or wound-dressing agents.

P15

ASSOCIATIONS OF SERUM GAMMA-LINOLENIC ACID LEVELS WITH ERYTHEMA SEVERITY AND ANXIETY/DEPRESSION STATUS IN PATIENTS WITH ROSACEA

Jin-Yi Tang[1#], Jian Li[1]*

1. Dermatology Department, Southwest Hospital, Third Military Medical University, Chongqing, China.
Correspondence to Jian Li (leejian860@126.com)

Abstract

Background The development of rosacea is suggested to be closely associated with lipid metabolism, inflammation, and anxiety/depression. Gamma linolenic acid (GLA) is a key factor participating in lipid metabolism, which is also confirmed to regulate the inflammatory response. However, the associations of serum GLA levels with rosacea severity and psychological status still remain unclear.

Methods A total of 62 rosacea patients were consecutively recruited. Patient's Self-Assessment (PSA) scale and clinician erythema assessment (CEA) as well as 7-item Generalized Anxiety Disorder (GAD-7) and 9-item Patient Health Questionnaire (PHQ-9) were conducted to evaluate the degree of erythema severity and anxiety/depression, respectively. Serum GLA levels were determined by gas chromatography-mass.

Results Lower levels of serum GLA in rosacea patients were observed ($P<0.001$), and subgroup analysis revealed that patients with higher-level GLA had lower scores of PSA, CEA, GAD-7 as well as PHQ-9 scores. Moreover, Pearson correlation analysis uncovered that serum GLA levels were negatively associated with PSA, CEA, GAD-7 as well as PHQ-9 scores, respectively. Linear regression model found that serum GLA levels at baseline were a predictive factor for prognosis of clinical outcomes after 1-month conventional treatment.

Conclusion Our study indicates that lower levels of serum GLA in rosacea patients are negatively associated with the degree of erythema and anxiety/depression status.

P16

EXTRACTION OF SKIN INTERSTITIAL FLUID BY SKIN STRETCHING USING A NOVEL 3D-PRINTED DEVICE

D. Tasdemir [1,2], M. Aly [1], F. Benaouda [1], T. Liao [2], G. R. McClelland [2], S. A. Jones [1,2]

[1]*Pharmaceutical Science, King's College London, Faculty of Life Science & Medicine, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom*
[2]*Centre for Pharmaceutical Medicine Research, King's College London, Faculty of Life Science & Medicine, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom*

Skin interstitial fluid (ISF) is a bodily fluid that transfers nutrients to cells and removes waste products. The composition of blood and ISF are similar in that they contain many of the same analytes, but in addition, because of its link with the lymphatic system, the ISF also contains numerous distinct biomarkers (1,2). As a result, ISF is a potentially clinically useful bodily fluid for disease prognostic and diagnostic purposes (2). However, conventional ISF sampling techniques are invasive, uncomfortable for participants and time-consuming, and this limits the use of ISF in research and clinical settings (3,4). There is a need for a simple ISF sampling method and the identification of useful ISF biomarkers. This study aimed to develop a method to extract ISF. Human ISF samples were obtained from 8 healthy volunteers by application of hypobaric pressure using a novel suction device at -310 mbar for 10 min, and this was compared to the same process using atmospheric conditions as a negative control. Protein and sodium levels were measured in extracted ISF by BCA assay and sodium probe. The mean amount of protein in ISF was 9.44 ± 6.18 μg and 19.17 ± 3.92 μg, and sodium concentration in ISF was 0.61 ± 0.41 mM and 1.39 ± 0.61 mM under atmospheric and hypobaric pressure. As shown in figure 1, there was statistical significance for the extracted amount of protein and sodium between under atmospheric pressure and hypobaric pressure (p: 0.0028 and p: 0.0119, unpaired t-test).Protein and sodium levels were used to calculate the extracted amount of ISF. The average volume of ISF collected using sodium as the marker was 0.87 ± 0.19 μL while using protein it was 0.47 ± 0.10 μL. This study showed that ISF can be collected by skin stretching and that both large and small molecular weight biomarkers can be detected in the extracted fluids.

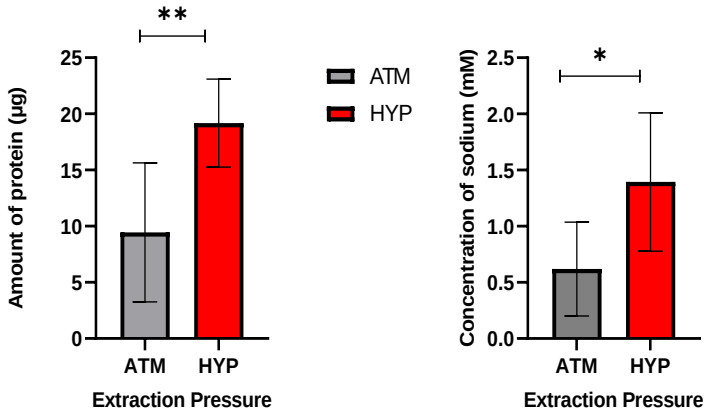


Figure 1. The average amount of protein (μg) and concentration of sodium (Mm) in extracted ISF under atmospheric conditions (ATM) and hypobaric pressure (HYP) for 10 min.

References;

1. Samant et al., *Science Translational Medicine*, 2022. *12*(571), eaaw0285.
2. Samant et al., *Proceedings of the National Academy of Sciences*, 2018. *115*(18), 4583–4588.
3. Miller et al., *Communications Biology*, 2018*:*1(1).
4. Inacio et al. *Journal of Controlled Release*, 2016: *226*, 66–76

P17

COMBINATION TREATMENT OF DAYLIGHT PHOTODYNAMIC THERAPY (DL-PDT) AND ALPHA-HYDROXY ACID(AHA) 30% CHEMICAL PEELS AS A NOVEL EFFECTIVE TREATMENT FOR PAPULOPUSTULAR ROSACEA

Jinyu Wei[1] , Jian Li [2]*

[1] The 920th Hospital of Joint Logistics Support Force of PLA, Kunming, 650100, China;
[2]Southwest Hospital, The Third Military Medical University, Chongqing, 400038, China.
**Correspondence to Jian Li (leejian860@126.com)*

Introduction: Papulopustular rosacea (PPR) is characterized by central facial erythema and papules and/or pustules, with or without telangiectases. Traditional treatment for PPR has been challenged due to systemic side effects or frequent relapses, which necessitates new treatments. Preliminary evidence showed that conventional photodynamic therapy (c-PDT) is an efficient therapy in treating PPR, especially for severe PPR, but the pain and severe post-inflammatory hyperpigmentation(PIH) are in urgent need of improvement, while the use of daylight photodynamic therapy (DL-PDT) and AHA 30% chemical peels of rosacea has been largely absent from the literature.

Objectives: The aim of this study was to compare the efficacy and safety of combination of DL-PDT using 5-aminolevulinic acid (ALA) and AHA 30% chemical peels and minocycline in the treatment of PPR. To identify which treatment have the largest or fastest impact on central facial erythema and papules and/or pustules.

Materials and Method: A total of 52 PPR patients were randomly assigned (22 :30) to receive DL-PDT using 5-ALA at 2-weeks intervals for three sessions and followed by alpha-Hydroxy acid 30% chemical peels at 2-weeks intervals for two sessions (group-1) or oral 50mg/day minocycline for 8 weeks (group-2), with 2 weeks of follow-up. DL-PDT treatment protocol involves the application of a photosensitizing agent without occlusion and subsequent exposure to ambient daylight within 30 min, with patients exposed to daylight for 1.5-2.0 h. The main outcomes were measured by skin lesion scores, physician's global assessment (PGA), CT Inflammation assessment of skin lesion. The secondary outcomes were measured by post-acne hyperpigmentation index (PAHPI) , scores of pain/sting, burning, itching, dermatology life quality index (DLQI) and generalized anxiety disorder (GAD-7).

Results: After treatment, patients in the two groups showed much lower skin lesion scores than before treatment, and there was no significant difference in the total efficacy among the two groups ($P > 0.05$). DL-PDT was highly effective for severe rosacea, achieving complete response (over 90% improvement) in 10 patients (10/22). DL-PDT did not significantly increase the pain/stinging of the patients. Notably, we found group-1 outperformed the group-2 in the comparison of the PIH levels ($P < 0.05$).

Conclusions: This study demonstrates that combination treatment of DL-PDT using ALA and AHA 30% chemical peels is an effective treatment with minimal side effects for papulopustular rosacea, so it is worthy of clinical promotion and application.

P17

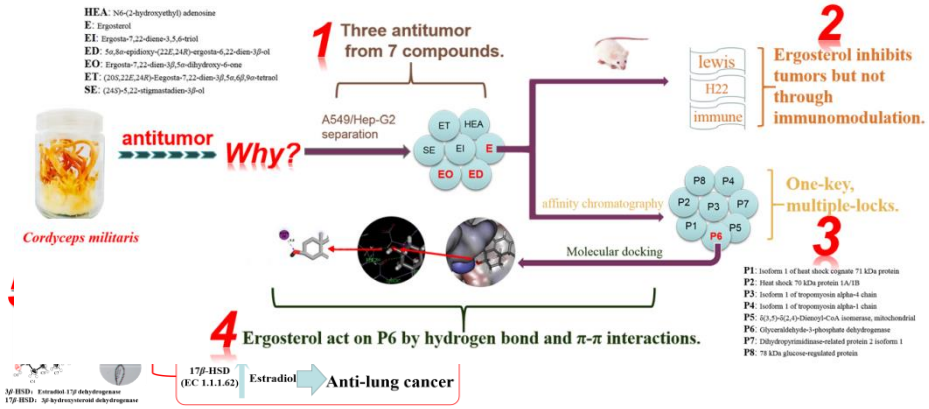
STUDY ON ANTICANCER MECHANISM OF METABOLIC ENZYME MUTATION OF ERGOSTEROL SINGLE CRYSTAL FROM CORDYCEPS MILITARIS.

Yujiao Chen[1,2,3], Wei Wang[4], Xiaoping Huang[4], Huawen Liu, Yuqian Wu[3], Min Chen[3], Zhenbo Liu[2], Jun Cao[3,*], Guixue Wang[1,*], Julia Li Zhong[1,*]

1. Key Laboratory for Biorheological Science and Technology of Ministry of Education, State and Local Joint Engineering Laboratory for Vascular Implants, Bioengineering College of Chongqing University, Chongqing, 400030, China.
2. Guizhou Aerospace Intelligent Agriculture Co. Ltd., Guizhou, 057250, China.
3. Guizhou Gui'an Academy of Precision Medicine Co. Ltd., Gui'an, 561113, China.
4. Oncology Centre, Three Gorges Hospital of Chongqing University,Wanzhou, Chongqing, China

The fruiting body of Cordyceps militaris Haining strain (C. militaris HN) antitumor compound in vivo and vitro, new conformational ergosterol, (3R,9R,10S,13S,14S,17S)-17-((2S,5R,E) –5,6-dimethylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,9,10,11,12,13,14,15,16,17-dodecahydro-1 -H-cyclopenta[α]phenanthren-3-ol), showed that its antitumor effect was not related to the improvement of immunity. Furthermore, detected the gene mutation of the metabolic enzymes by SMRT(single molecular real-time) sequencing, its inhibition of A549 was related to 17β-HSD, but the inhibition of HepG2 was related to 3β-HSD. This study indicate that the C.militaris anticancer mechanisms mainly involved multiple interactions with targets and pathways. And the ergosterol single crystal from C. militaris HN fruiting body specifically inhibited cancer by regulating the 17β-HSD in A549 and reducing the 3β-HSD in HepG2. This may provide an important metabolic new target to treat lung cancer.

Acknowledgements: The Fundamental Research Funds for the Central Universities (Project No. 2022CDJYGRH-005&2022CDJYGRH-016 to JL Zhong); Ling Hui (Guangzhou) Biotech Co. Ltd. for scientific conference, travel and review expenses of JL Zhong. Wu Jieping foundation to W Wang (320.6750.2022-22-38): Real world study on the efficacy and safety of serplulimab combined with EC in first-line treatment of extensive small cell lung cancer. The 111 Project of Biomechanics and Tissue Repair (B06023).



P18

MICRO-INJURY INDUCES HAIR REGENERATION AND VITILIGO REPIGMENTATION THROUGH WNT/B-CATENIN PATHWAY.

Wei Xu[1]*, Xiaofeng Han[1], Yadan Ding[2], Li Yang[2], Julia Li Zhong[2], Mao Lin[1], Qingchun Diao[1]

*E-mail: davidweixucn@gmail.com (Poster)

[1]Department of Dermatology, Chongqing Hospital of Traditional Chinese Medicine, Chongqing, China; [2]Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, China

Abstract: Extrinsic injury can evoke intrinsic stimulation and subsequently initiate the physiological repair process. This study aims to investigate whether clinically acceptable micro-injury could be used to create local stimuli to induce hair regeneration and vitiligo repigmentation. We discover that proper micro-injury effectively induces hair regeneration by activating the hair follicle stem cell proliferation and migration downwards to the hair matrix, finally shifting the hair follicle stage from telogen into anagen. On vitiligo model mice, micro-injury also induces the hair follicle melanocyte stem cells to migrate upwards to the interfollicular epidermis, activating and giving rise to melanocytes to repopulate the vitiligo lesion. Mechanistic analysis indicates that the canonical Wnt/β-catenin pathway plays a key role in the micro-injury-induced repair process. This study demonstrates that micro-injury has great potential in inducing hair regeneration and vitiligo repigmentation, laid a foundation to develop a micro-injury-based treatment method in alopecia and vitiligo. We Conclude that proper micro-injury effectively promotes hair regeneration and vitiligo repigmentation. The mechanism probably involves mobilizing the HF-SCs to migrate downwards to the hair matrix to contribute to hair regeneration, as well as the HF-McSCs to migrate upwards to the interfollicular epidermis to repopulate vitiligo melanocytes (Fig. 3E). The Wnt/□-catenin pathway plays a key role in micro-injury induced recovery. This study lays a foundation to develop a micro-injury-based treatment method in alopecia and vitiligo.

Acknowledgements: This work was supported by the NSFC, China (81503588, 82074437) and the Natural Science Foundation of Chongqing (cstc2019jcyj-msxmX0741, 2020ZY013906, CQSZYY2020011), The Fundamental Research Funds for the Central Universities (Project No. 2022CDJYGRH-005 to JL Zhong) and the 111 Project of Biomechanics and Tissue Repair (B06023) to Li Yang.

P19

ERIODICTYOL PROTECTS SKIN CELLS FROM UVA IRRADIATION-INDUCED PHOTODAMAGE BY INHIBITION OF THE MAPK SIGNALING PATHWAY

Muhammad Farrukh Nisar[1], Shizhuang Lin[2], Tiantian Liu[1], Wei Wang[1], Li Yang[1], Jing Yang[1], Julia Li Zhong*.

[1]*Bioengineering College & Dermatology, Three Gorges Hospital, Chongqing University, Chongqing 400044, PR China,*

[2]*Department of the Dermatology, The First People's Hospital of Wenling City, Zhejiang Province*

Abstract: Chronic exposure of solar UVA radiation to human skin may aid the accumulation of excessive and persistent levels of ROS that imbalances the redox status of the cells and initiate pro-inflammatory factors. UVA mainly shows its effects in the skin by generating ROS that imbalance the redox homeostasis in the skin cells. The latter can be modified and protected by activation of natural built-in antioxidant enzymes such as nuclear factor erythroid 2-related factor 2(Nrf2)/heme oxygenase 1 (HO-1) pathway in human skin cells. In the present study, we evaluated the photoprotective effects of eriodictyol on skin cells (HaCaT keratinocytes and FEK4 fibroblasts) against the UVA radiation-induced oxidative damage by monitoring the expression of inflammatory factors and MMP-1 in vitro. We also examined how eriodictyol affects expression levels of MMP-1, TIMP-1, COL-1, and phosphorylation states of MAPKs (JNK, ERK, p38) in UVA-irradiated skin cells. This study showed that eriodictyol has protective effects in both HaCaT and FEK-4 cells subjected to UVA irradiation, through enhancing cell proliferation, reducing the intracellular ROS generation and downregulating the expression of inflammatory factors and MMP-1, but upregulating the expression of Timp1 and Col1. The eriodictyol can be considered as a potential drug to confer photoprotection. Further study of both eriodictyol and AKBA in protection of tissue injury are currently on the way.

Acknowledgements: Science and Bureau of Finance Joint medical research project in Wenling District of Zhejiang to SZ Lin. Science and Health Joint medical research project in Chongqing Wanzhou District (wzstc-kw2020007). Ling Hui (Guangzhou) Biotech Co. Ltd. for scientific conference, travel and review expenses of JL Zhong. The Fundamental Research Funds for the Central Universities (Project No. 2022CDJYGRH-005; 2022CDJYGRH-016 to JL Zhong) and the 111 Project of Biomechanics and Tissue Repair (B06023) to Li Yang.

P19

BACH2 REGULATES AUTOPHAGY TO MODULATE UVA-INDUCED PHOTOAGING IN SKIN FIBROBLASTS

E-mail: wangmei5108@126.com

Mei Wang[1,2], ChuanfengYang[3], Yongsheng Yao[3]， Yujiao Chen[1], Zheng Chen[3] , Li Yang[1], Julia Li Zhong[1,3]

[1]National Innovation and Attracting Talents "111" base, Key Laboratory of Biorheological Science and Technology of Ministry of Education, Bioengineering College, Chongqing University, Chongqing, 400044, China; [2]Key Laboratory of Basic Pharmacology of Ministry of Education and Joint International Research Laboratory of Ethnomedicine of Ministry of Education, Zunyi Medical University, Zunyi, 563000, China.
[3]Department of Dermatology. SapingBa People's Hospital of Chongqing University

Abstract: Senescence is a cellular process that can be initiated by certain stressors such as UVA irradiation. Chronic exposure of skin fibroblasts to UVA resulted in a significant decrease in Bach2 expression, both in vitro and in vivo. In addition, knockdown of Bach2 in skin fibroblasts led to an increased expression of cell senescence-related genes, which further enhanced the UVA -induced photoaging. The overexpression of Bach2 resulted in a decrease in the expression of cell senescence-related genes and the knockdown of Bach2 in skin fibroblasts can lead to a decreased expression of autophagy-related genes and vice versa, suggesting that autophagy is involved in Bach2-mediated regulation of senescence in skin fibroblasts. Additionally, inhibition of autophagy with 3-MA suppressed the expression of autophagy-related proteins and promoted cell senescence. Furthermore, knockout of Atg5 or Atg7 in embryonic mouse fibroblasts led to a significant increase in the expression of cell senescence related genes. Immunoprecipitation assays further demonstrated that Bach2 directly interacts with Beclin-1, Atg3, Atg7, and LC3 in fibroblasts. This study demonstrated that Bach2 modulates the extent of UVA-induced photoaging through regulation of autophagy in skin fibroblasts. Further in depth understanding the function in both skin fibroblasts and keratinocytes are currently under investigation.

Acknowledgements: The National Natural Science Foundation of China (8226140585); Science and Technology Plan Project of Guizhou Province ZK [2021]565; The Fundamental Research Funds for the Central Universities (Project No. 2022CDJYGRH-005 & 2022CDJYGRH-016 to JL Zhong) ; Ling Hui (Guangzhou) Biotech Co. Ltd. for scientific conference, travel and review expenses of JL Zhong. The 111 Project of Biomechanics and Tissue Repair (B06023) to Li Yang.

P20

KLK5/PAR2 CONTRIBUTED TO THE RECRUITMENT OF CYTOTOXIC CD8+ T CELLS VIA ACTIVATION OF JAK1/STAT1/CXCL 10 IN VITILIGO EPIDERMAL KERATINOCYTES.

Fujin Yang, Qingchun Diao, Mao Lin*.*E-mail:lmsleeper@aliyun.com.

Department of Dermatology, Chongqing Hospital of Traditional Chinese Medicine, Chongqing.

Vitiligo is an acquired, progressive depigmenting disorder characterized by the loss of functional melanocytes from the epidermis. KLKs are well-known as enzymes disconnecting corneum cells in epidermis. But the role of KLKs in vitiligo has not been well-understood. We reported the decrease level of both KLK5 and receptor Par2 in vitiligo skin. Furthermore, we've found that the decreased KLK5/Par2 triggered the JAK/STAT/CXCL 10 signaling and inhibited the Nrf2/HO-1 pathway in kerationcytes. Thus, as a cross point on the uptream of auto-immune signaling jak/stat/cxcl 10 and the antioxidant signaling Nrf2, KLK5/PAR2 maybe one of the key factors in pathogenesis of vitiligo. KLK5/Par2 may be a therapeutic target for the treatment of vitiligo.

Acknowledgements: Chongqing Medical Scientific Research Project (Joint project of Chongqing Health Commission and Science and Technology Bureau) (2022DBXM007)

P21

THE REDUCTION OF COLLAGEN SECRETION BY FOXP3 IN KELOID FIBROBLASTS [E-mail: 1753143630@qq.com]

Yumeng Deng, Ruiyan Cen, Liqun Wang, Yan Zhao, Xia LEI.

Department of Dermatology, Daping Hospital,Chongqing,400010,China.
Department of Dermatology, the Third Affiliated Hospital of Chongqing Medical University,Chongqing 401120,China.

Excessive collagen deposition and persistent local inflammation are characteristic of keloids, but there are no effective therapies to inhibit both excessive collagen secretion by fibroblasts and suppress the persistent local inflammatory response, in which the TGF-β/SMAD pathway may play a major role.

Here we first observed the difference between the keloid tissue group (keloid) and normal skin tissue (Con) and confirmed that the levels of COL1, COL3, IL-6, and TGF-β were elevated and the levels of FOXP3 were decreased in keloid tissue. Next, we verified that TGF-β and FOXP3 factors expression in keloid fibroblasts (KFB) by cellular IHC immunohistochemistry. Using *FOXP3 overexpression adenovirus* in the cells (CON and Ad-FOXP3), ELISA, WB were used to detect the expression levels of COL1, COL3, IL-6, TGF-β, and we confirmed the decrease of COL1, COL3, IL-6, TGF-β expression in the presence of FOXP3 overexpression, indicate the reversed relationship. Then TGF-βinhibitor: LY2157299 treatment causes the same result as overexpression of FOXP3 i.e. overexpression of FOXP3 cause a decrease of TGF-β、COL1, COL3, IL-6, level. We concluded that overexpression of FOXP3 could inhibit collagen secretion from KFB and suppress IL-6 expression by affecting TGF-β. Whether it can affect TGF-β/SAMD, a pathway that plays an important role in keloid pathogenesis, remains to be further investigated.

Acknowledgements: National Natural Science Foundation of China：81773348