ipbs TOULOUSE CANCER INFECTION INFLAMMATION

EMBO

EMBO Workshop

Mechanisms of Citrullination Regulation in Health and Disease

About The Event

We aim to promote scientific knowledge exchange in the field of protein citrullination, an essential post-translational modification. We will discuss emerging concepts in cancer, inflammation, autoimmunity and tissue homeostasis, fibrosis and regeneration.

Organizers

Priyanka Sharma
Institute of Pharmacology and Structural Biology, FR

Francoise Viala (Admin)
Institute of Pharmacology and Structural Biology, FR

Isabelle Saves (Admin)
Institute of Pharmacology and Structural Biology, FR

Co-organizers

Maria Christophorou
The Babraham Institute, UK

Michel Simon
Toulouse Institute for Infectious and Inflammatory Diseases, FR

Registration

Abstract submission deadline
21 May 2024

Student/Postdocs
(in-person/virtual)..... 350/150 EUR
Academic
(in-person/virtual)..... 550/300 EUR
Industry
(in-person/virtual)..... 850/500 EUR

Speakers

Brian Cherrington
University of Wyoming Laramie, US

Maria Christophorou
The Babraham Institute, UK

Marco De Andrea
University of Turin Medical School, IT

Maria Genander
Karolinska Institutet, SE

Sergei Grigoryev
Penn State College of Medicine, US

Jyrki Heino
University of Turku, FI

I-Cheng Ho
Brigham and Women's Hospital Harvard Medical School, US

Anna Huttenlocher
University of Wisonsin, US

Mariana Kaplan
National Institutes of Health, US

Xisong Ke
Shanghai University of Traditional Chinese Medicine, CN

Sigrun Lange
University of Westminster, UK

Kimberly Martinod
Centre for Molecular and Vascular Biology, BE

Avinash Padhi
Karolinska Institutet, SE

Ger Pruijn
Radboud University, NL

Marco Radic
University of Tennessee Health Science Center, US

Guy Serre
Toulouse Institute for Infectious and Inflammatory Diseases, FR

Priyanka Sharma
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Miriam Shelef
University of Wisconsin-Madison, US

Michel Simon
Toulouse Institute for Infectious and Inflammatory Diseases, FR

Nicholas Thangue
Oxford University, UK

Paul Thompson
UMass Chan Medical School, US

Louise Walport
The Francis Crick Institute, UK

Xuoshang Xu
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Day 1 - July 9, 2024

14:00 Opening

14:30-15:30 **Functional role of citrullination in human diseases - Paul Thompson - UMass Chan Medical School, US KEYNOTE TALK**

15:30-16:00 BREAK/ SOCIAL EVENT Coffee break - Meet the Speaker

AUTOIMMUNITY AND RHEUMATOLOGY

16:00-16:30 **Flying over 40 years of research on anti-citrullinated protein antibodies: diagnosis, pathophysiology and specific immunotherapy of Rheumatoid Arthritis** - Guy Serre - *Toulouse Institute for Infectious and Inflammatory Diseases, FR*

16:30-17:00 **Citrullination regulates autoimmunity** - Marianna Kaplan - *National Institutes of Health, US*

17:00-17:30 **Rapid detection of anti-citrullinated protein antibodies in autoimmune patients** - Ger Pruijn - *Radboud University, NL*

17:30-18:00 BREAK/ SOCIAL EVENT Coffee break - Meet the Speakers

NEUTROPHIL EXTRACELLULAR TRAPS

18:00-18:30 **PADI4 function in Immunothrombosis -** Kimberly Martinod - *Centre for Molecular and Vascular Biology, BE*

18:30-19:00 **Functional role of citrullination in cancer** - Nicholas La Thangue - *Oxford University, UK*

19:00-19:30 **PADIs and Citrullination in Extracellular Traps, Autoantibodies, and Rheumatoid Arthritis -** Miriam Shelef - *University of Wisconsin-Madison, US*

19:30-22:00 BREAK/ SOCIAL EVENT Dinner and Meet the Speakers

Day 2 - July 9, 2024

DEVELOPMENT, TISSUE BIOLOGY AND REGENERATION I

09:00-09:30 **PADI4 in the regulation of cell fate transitions** - Maria A. Christophorou - *The Babraham Institute, UK*

09:30-10:00 **Citrullination dynamics in stem cell lineage progression -** Maria Genander - *Karolinska Institutet, SE*

10:00-10:15 **Cardiac PAD2 Expression and Myocardial Citrullination Decline with Age in Female Mice Independent of Estrogen -** Samantha Shorthill, *University of Wyoming, US*

10:15-10:30 **Untangling a possible catalytic function of PADI6 in early embryo development -** Jack Williams, *Imperial College, London, UK*

10:30-11:00 **Histone citrullination in tissue regeneration and repair -** Anna Huttenlocher - *University of Wisonsin, US*

11:00-11:30 BREAK/ SOCIAL EVENT Coffee break - Meet the Speakers

DEVELOPMENT, TISSUE BIOLOGY AND REGENERATION II

11:30-12:00 **Gonadotrope Cells Contribute to Subfertility in Female Pad2/Pad4 Double Knockout Mice -** Brian Cherrington - *University of Wyoming Laramie, US*

12:00-12:30 **Peptidylarginine deiminases and deiminated proteins at the epidermal barrier -** Michel Simon - *Toulouse Institute for Infectious and Inflammatory Diseases, FR*

12:30-13:00 **PADIs in skin inflammation -** Avinash Padhi - *Karolinska Institutet, SE*

13:00-13:30 **PADI-mediated modulation of extracellular vesicle signatures -** Sigrun Lange - *University of Westminster, London, UK*

13:30-15:00 BREAK/ SOCIAL EVENT Lunch and Meet the Speakers

CANCER

15:00-15:30 **Citrullination targets cancer stem cells -** Xuesen Zhang - *Nanjing Medical University, CN*

15:30-15:45 **PADI4 regulates p53-mediated tumor suppression and post-translationally modifies p53 via citrullination** - Alexandra Indeglia - *The Wistar Institute, US*

Day 2 - July 9, 2024

15:45-16:00 **PAD4 controls tumor immunity via restraining MHC-II machinery in macrophages** - Michael Pitter - *University of Michigan School of Medicine, US*

16:00-16:15 **Citrullination of fibrinogen creates a pre-metastatic site to facilitate lung metastasis in cancer patients -** Takeshi Tomita - *Shinshu University School of Medicine, JP*

16:15-16:30 **PADI inhibition overcomes the acquisition of resistance after KRAS blockage in pancreatic ductal adenocarcinoma cells -** Francesca Agostini - *University of Udine, IT*

16:30-16:45 **Quantifying Neutrophil Extracellular Trap Release and Citrullinated Histone H3 in an Infection-Inflammation NET - Array Microsystem-** Caroline N Jones - *University of Texas Southwestern medical center Dallas, Dallas, US*

16:45-17:00 BREAK/ SOCIAL EVENT Coffee break

FLASH TALKS & POSTERS

17:00-18:00 Flash Talks (5 minutes) for Poster session I

A novel mass-spectrometry-based approach enabling site-specific detection of plasma protein citrullination in inflammation-induced thrombosis , Jan Voorberg, Sanquin Research, NL

Exploring Motif Preferences and Functional Specificity of the Protein Arginine Deiminase Family, Sophia Laposchan, University of Munich, Germany

Development of a Mass Spectrometry-Compatible Chemical Probe for Protein Citrullination Enrichment, Rebecca Meelker Gonzalez, Technical University of Munich, Germany

Peptidylarginine deiminase 4 (PAD4) is a key factor for SARS-CoV-2 replication and SARS-CoV-2 induced pro-inflammatory responses, Selina Pasquero, University of Turin, IT

Investigating PAD-mediated citrullination in HPV-driven head and neck squamous cell carcinoma, Camilla Albano, University of Turin, IT

PAD3 autocitrullination and the structure, Masaki Unno, Ibaraki University, JP

Down-regulation of human PAD1 in a three-dimensional reconstructed epidermis affects nucleophagy and epidermal barrier function. Marie-Claire Méchin, Toulouse Institute for Infectious and Inflammatory Diseases, FR

18:15-19h15 Poster session I

Day 3 - July 10, 2024

CHROMATIN AND TRANSCRIPTION

09:30-10:00 **Citrullination dictates the transcriptional event -** Priyanka Sharma - *Institute of Pharmacology and Structural Biology, FR*

10:00-10:30 **Unfolding of chromatin condensates by PAD4-mediated histone citrullination -** Sergei Grigoryev - *Penn State College of Medicine, US*

10:30-10:45 **Citrullinating enzyme PADI4 and transcriptional repressor RING1B bind in cancer cells -** Jose Luis Neira - *Universidad Miguel Hernández, Elche, SP*

10:45-11:30 BREAK/ SOCIAL EVENT Coffee break - Meet the Speakers

CHEMICAL AND STRUCTURAL BIOLOGY TOOLS FOR CITRULLINATION

11:30-12:00 **Exploring PADI regulation using cyclic peptide tools -** Louise Walport - *The Francis Crick Institute, UK*

12:00-12:30 **Identification of a small molecule activating histone citrullination-** Xisong Ke - *Shanghai University of Traditional Chinese Medicine, CN*

12:30-12:45 **Non-protein activators of PAD4** - Tomasz Kantyka - Jagiellonian University, Krakow, P

12:45-13:00 **Deep Learning Boosts Citrullination Identification in Mass Spectrometry-Based Proteomics** - Chien-Yun Lee - Technical University of Munich, Germany

13:00-14:30 BREAK/ SOCIAL EVENT - Lunch and Meet the Speakers

EXTRACELLULAR FUNCTIONS OF CITRULLINATION

14:30-15:30 **Uncovering nuclear expulsion of tumor cells: mechanisms of apoptosis and metastatic outgrowth - Li Yang- Center for Cancer Research, NIH, US**
KEYNOTE TALK

Day 3 - July 10, 2024

15:30-16:00 **Intrinsic roles of PAD2 in fibroblasts** - I-Cheng Ho - *Brigham and Women's Hospital Harvard Medical School, US*

16:00-16:30 **Citrullination in the regulation of the extracellular matrix** - Jurki Heino - *University of Turku, FI*

16:30-16:45 BREAK/ SOCIAL EVENT Coffee break

FLASH TALKS & POSTERS

16:45-17h45 Flash Talks (5 minutes) for Poster session II

Deimination of filaggrin improves its proteolysis in the epidermis, Marie-Claire Méchin, Toulouse Institute for Infectious and Inflammatory Diseases, FR

Non-cell-autonomous regulation of cell reprogramming through extracellular citrullinated chromatin, Johanna Grinat, The Babraham Institute, UK

Structural insight into the function of human peptidyl arginine deiminase 6, Jack Williams, Imperial College London, UK

Understanding histone citrullination as an epigenetic modulator, Noah Shriever, The Babraham Institute, UK

Susceptibility of Complement system proteins on citrullination with human PAD4, Ewa Bielecka, Jagiellonian University, Krakow, P

Inhibitors of peptidyl-arginine deiminases and histone citrullination decrease the angiogenic potential and Akt activation in human endothelial cells, Oskar Ciesielski, University of Lodz, P

Exploring the functions of histone citrullination in modulating stem cell behaviour, Hin Man Mak, Karolinska Institutet, SE

Elucidating the role of PADI4 in p53-mediated tumor suppression, Andrea Valdespino, The Wistar Institute, US

17:45-19h00 Poster session II

19:30-21:30
BREAK/ SOCIAL EVENT
Boat Trip / Dinner
Meet the Speakers

21:30-23:00
Networking Drinks

Day 4 - July 11, 2024

PHYSIOLOGY AND DISEASES

09:00-09:30	**Protein Citrullination in Amyotrophic Lateral Sclerosis -** Zuoshang Xu - *University of Massachusetts Chan Medical School, US*
09:30-10:00	**Virus-induced citrullination as a strategy to subvert the host's innate antiviral defence** - Marco De Andrea - *University of Turin Medical School, IT*
10:00-10:30	**Shifting neutrophils into gear, the role of cytoskeletal citrullination -** Marko Radic - *University of Tennessee Health Science Center, US*
10:30-11:00	BREAK/ SOCIAL EVENT Coffee break - Meet the Speakers
11:00-12:00	Panel Discussion
12:00-12:30	Poster and Oral Presentation Awards, granted by the Toulouse Cancer Santé foundation. Closing Remarks
12:30-13:00	Packed Lunch and Departure

Invited speakers

Brian Cherrington

University of Wyoming Laramie, US

Gonadotrope Cells Contribute to Subfertility in Female Pad2/Pad4 Double Knockout Mice

Anterior pituitary gland gonadotrope cells synthesize and secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are required for follicle development and subsequent ovulation in females. In mice, PAD expression is highest in gonadotrope cells during the estrus phase of the estrous cycle when ovulation occurs. In gonadotropes, histone citrullination regulates the expression of the LHβ subunit gene and the riboprotein DiGeorge Syndrome Critical Region 8 (DGCR8) microprocessor complex subunit, which is required for canonical miRNA biogenesis. PADs also localize to the gonadotrope cytoplasmic compartment where they citrullinate the cytoskeletal proteins actin and tubulin, which may contribute to hormone vesicle trafficking and secretion. Our recent work shows that female Pad2/Pad4 double knockout (Pad2/4 DKO) mice are subfertile. Specifically, DKO females have delayed pubertal onset, disrupted estrous cycles, and smaller uteri as compared to controls. In pituitaries from female Pad2/4 DKO mice, gonadotrope specific gene expression is altered as compared to estrous cycle matched wild type controls. Changes in gonadotrope gene expression and irregular estrous cycles suggest that the loss of Pad2/4 in pituitary gonadotropes contributes to subfertility in female DKO mice.

Brian Cherrington completed his undergraduate degree at Washington University in St. Louis and then received a M.S. and Ph.D. in reproductive endocrinology from Colorado State University. In his first post-doc at the University of California, San Diego he investigated gene programs in anterior pituitary gonadotrope cells. He then completed a second post-doc at Cornell University investigating the function of peptidylarginine deiminase (PAD) enzymes in the mammary gland and breast cancer. He joined the department of Zoology and Physiology at the University of Wyoming in 2011 and is currently an associate professor. His lab studies the function of PAD enzymes and citrullinated proteins in gonadotrope cells and the female reproductive tract.

Maria A. Christophorou

The Babraham Institute, UK

PADI4 in the regulation of cell fate transitions

The reprogramming of somatic cells to an induced pluripotent stem (iPS) cell state requires profound signalling, transcriptional and epigenetic rewiring. Similarly, the regeneration of mammalian tissues involves significant changes in cell behaviour and the transient acquisition of functions mediate the repair of damage and which can be thought of as adaptive cell reprogramming. We previously demonstrated that protein citrullination is induced upon the introduction of the Yamanaka transcription factors into somatic cells, where it precedes and mediates their reprogramming. We have since found that the induction of citrullination is a general, evolutionarily conserved feature of tissue regeneration. The cells that activate citrullination are mutually exclusive with the iPS or tissue stem cells, suggesting that they act as “active bystanders” that mediate reprogramming in a non-cell-autonomous manner. The bystander cells release citrullinated chromatin to the extracellular space, in a process akin to Neutrophil Extracellular Traps. Blocking of extracellular chromatin components reduces reprogramming, as does pharmacological interference with extracellular chromatin-sensing signalling pathways, while ablation of the citrullinating enzyme PADI4 impairs regeneration. Our findings open a new research avenue into the study of citrullination and extracellular chromatin as a cell communication mechanism that mediates cell fate transitions.

Maria Christophorou is a Principal Investigator in Epigenetics at the Babraham Institute in Cambridge, UK. Her lab studies the biochemical mechanisms that modulate the function of epigenetic regulators, focussing primarily on protein citrullination. She studied Biology at MIT as a Fulbright Scholar and completed a PhD at UCSF, where she studied mechanisms of p53-mediated tumour suppression. As a postdoc, she focussed on chromatin biology at The Gurdon Institute, Cambridge University, where she discovered that the peptidylarginine deiminase PADI4 regulates pluripotency and described a new molecular mechanism via which PADI4 mediates chromatin decondensation. She started an independent group as a Wellcome Trust and Royal Society Sir Henry Dale Fellow and Wellcome-Beit Fellow at the University of Edinburgh, before moving to Babraham Institute. Maria is passionate about bringing the citrullination research community together. She organised the first international conference in the field in 2022 and is a co-organiser of this meeting.

Marco De Andrea
University of Turin Medical School, IT

Virus-induced citrullination as a strategy to subvert the host's innate antiviral defense

One of the strategies developed by a number of bacteria and viruses to favor their replication consists in modifying host cellular proteins at the post-translational level, thereby altering their localization, interaction, activation and/or turnover. Among these, the post-translational modification (PTM) named citrullination/deimination is the process where the guanidinium group of an arginine is hydrolyzed to form citrulline, a non-genetically encoded amino acid. This PTM is catalyzed by the calcium-dependent protein arginine deiminase (PAD) family of enzymes, which in humans is composed of five isoforms (PADs 1-4 and 6), with different tissue-specific expression and substrate specificities. Although aberrant citrullination has been detected in several inflammatory conditions, suggesting that it may play a pathogenic role in inflammation-related diseases, a direct correlation between citrullination and viral infections has only recently emerged. Here, I'll describe the more recent results from our group that led to the definition of the citrullination profile, the specific expression of different PADs, and the citrullinated substrates in the course of infections with both DNA and RNA viruses. In addition, the results obtained with a large panel of PAD inhibitors, describing their antiviral activity against viruses from the *Herpesviridae* and *Coronaviridae* families in a wide variety of cell lines, will be discussed.

Marco De Andrea, MD, PhD, is currently Associate Professor of Medical Microbiology at the University of Turin (Medical School, Italy), and Head of the Intrinsic Immunity Unit in the Center for Translational Research in Autoimmune and Allergic Diseases (CAAD Center, Novara Medical School, Italy). He is also Founder and head of the Scientific Board of NoToVir srls, a biotech start-up established on December 2017, active in the field of medical biotechnology, whose main objective is the development and screening of innovative antiviral molecules.
His scientific activity, documented by more than 100 publications, can be summarized as follows:

- *Characterization of the post-translational modification citrullination upon viral infections in order to develop new host-targeting antivirals (HTAs);*
- *Unveiling the role of PYHIN proteins in the onset of inflammation and progression to autoinflammation;*
- *Study of the role of the IFI16 protein as a DNA/RNA sensor and novel restriction factor against viral replication.*

Maria Genander

Karolinska Institutet, SE

Citrullination dynamics in stem cell lineage progression

Stem and progenitor cell state transitions are closely controlled to enable tissue formation. How PADIs and citrullination contribute to lineage progression is largely unknown. We find that PADI4 is dynamically regulated during hair follicle formation, and that PADI4 mRNA is enriched in a subset of progenitor cells in the hair bulb. Loss of PADI4 *in vivo* results in increased progenitor proliferation and retarded commitment to the hair shaft lineage, suggesting that PADI4 acts to balance progenitor proliferation and commitment to differentiation. Characterization of the PADI4-dependent citrullinome reveals protein targets linked to mRNA processing and translational control, and *in vivo* OP-Puro incorporation confirm that translation rates are increased in the absence of PADI4. Mechanistically, lack of PADI4 leads to phosphorylation of AKT and S6 whereas paradoxically, abundance of the downstream translational suppressor 4E-BP1 is reduced, suggesting a PADI4-dependent rewiring of the translational machinery. Collectively, our findings suggest a non-epigenetic role for PADI4 in orchestrating hair follicle lineage progression through fine-tuning of the translational landscape.

Maria Genander's group is interested in epithelial stem cell biology and how mechanisms maintaining epithelial homeostasis are changed during tumor initiation and repair. Maria has a PhD from Karolinska Institutet and performed her postdoctoral work at Rockefeller University. Maria's lab is using the squamous epithelia of the skin and esophagus to identify mechanisms that impact stem and progenitor cell behaviors, including the role of citrullination in stem cell lineage progression. In this meeting, Maria will discuss a non-epigenetic function of PADI4 in rewiring the translational landscape in hair follicle progenitor cells.

Sergei Grigoryev
Penn State College of Medicine, US

Unfolding of chromatin condensates by PAD4-mediated histone citrullination

Chromatin structure is subject to regulation by posttranslational histone modifications. One of the strongest effects is imposed by arginine citrullination of histone H3 and H4 N-tails by arginine deiminase PAD4, which mediates formation of neutrophil extracellular traps (NETs) in granulocytes. To study mechanism of chromatin unfolding by histone citrullination independent of proteases active in NETosis, we used reconstituted nucleosome arrays and native nucleosome arrays isolated from human myeloid cells. To reveal chromatin structure transitions, we applied Cryo-electron tomography combined with quantitative stereological analysis. With reconstituted chromatin, we observed a complete disruption of chromatin higher order structure by histone citrullination. In contrast, with native chromatin we observed a strong inhibition of nucleosome close-range interactions and dissolving of large nucleosome condensates with relatively little effect on the conformation of nucleosome chains. We propose that the main effect of PAD4-induced citrullination in native chromatin is to inhibit nucleosome close-range interactions by modifying the histone H3 and H4 tails. This structural transition may trigger NETosis by unfolding nucleosome condensates and opening them to proteases, that would further digest the histone tails leading to the complete nuclear rupture.
This work was supported by US NSF grant 1911940 to S. Grigoryev.

Sergei Grigoryev obtained his PhD in Molecular Biology from Lomonosov Moscow State University where he became fascinated with histones and epigenetic mechanisms altering chromatin structure and gene expression. After he came to the United States, he did a postdoctoral training at Caltech with Alex Varshavsky, a pioneer of targeted cellular proteolysis, where Sergei studied ubiquitin-mediated proteolysis using yeast molecular genetics. Later, he worked at the University of Massachusetts where he studied chromatin structure using molecular techniques and electron microscopy in close collaboration with Chris Woodcock, co-discoverer of nucleosome beads-on-a-string structure. Over the last 20 plus years at Penn State College of Medicine, Sergei's laboratory made several important advances towards understanding chromatin higher-order folding, mechanism of chromatin condensation by architectural chromatin proteins, and how do chromatin structure and epigenetic histone modifications mediate molecular mechanisms underlying cell differentiation, innate immunity, and cancer.

Jyrki Heino
University of Turku, FI

Citrullination in the regulation of the extracellular matrix

In chronic inflammation extracellular matrix (ECM) proteins are frequently citrullinated. We have shown that citrullination of ECM proteins, such as collagens and fibronectin, can also affect cellular interactions, e.g. integrin-mediated cell adhesion. In an analysis of 40 synovial fluid samples derived from chronically inflamed human joints we detected citrullination of 55 arginine recidues in extracellular proteins. Many of the sites had a characterized function related to the hallmarks of destructive joint inflammation. We have also extended our studied on citrullination of matrisome proteins in cancer. In this purpose we analyzed cancer proteomics data sets in 3 public databases for citrullinated matrisome proteins. While we detected citrullination of ECM, there was significant variation between tumors. Most frequently citrullinated proteins included fibrinogen and fibronectin, which are typically citrullinated in rheumatoid inflammation. We also detected correlation between immune cell marker proteins, matrix metalloproteinases and ECM citrullination, which suggests that in cancer, citrullination of matrisome proteins is predominantly an inflammation-related phenomenon.
In addition, we have developed three-dimensional cell culture methods to study ECM citrullination in experimental conditions.

Jyrki Heino defended his doctoral thesis in the University of Turku, Finland. A year later he joined professor Joan Massagué's group at the Department of Biochemistry, University of Massachusetts, Worchester, M.A., and later moved with the same group to the Cell Biology and Genetics Program, Sloan-Kettering Institute, New York, NY. After returning back to Finland Jyrki Heino established his own research group. Presently he is the professor and chair of biochemistry in the University of Turku and the director of the Department of Life Technologies. During the past 35 years Jyrki Heino and his research group have published several novel observations related to the biology, structure, evolution and biomedical role of extracellular matrix and cell adhesion receptors. Jyrki Heino is a member of the Finnish Academy of Science and Letters.

I-Cheng Ho

Brigham and Women's Hospital Harvard Medical School, US

Intrinsic roles of citrullination in fibroblasts

Citrullination, a unique form of posttranslational modification of proteins, is catalyzed by peptidylarginine deiminases (PADs). Recent studies have clearly demonstrated that citrullination critically regulates the function of immune cells, such as neutrophils, macrophages, and lymphocyte. However, it remains elusive as to whether citrullination also intrinsically regulates the function of non-immune cells, such as fibroblasts, which are the major source of extracellular matrix, and can functionally interact with immune cells. This talk will present data showing how unbiased multi-omic approaches lead to the discoveries of unexpected roles of PAD2 in regulating the formation of elastic fibers and the development of interstitial lung diseases/fibrosis through lung fibroblasts. In addition, the potential impacts of PADs through fibroblasts on emphysema and oncogenesis will be discussed. The audience is expected to gain a global view of how dysregulated citrullination in fibroblasts may contribute to the development of many human diseases.

Dr. I-Cheng Ho is currently a member of American Society of Clinical Investigation, an associate professor at Harvard Medical School, and a board-certified rheumatologist at Brigham and Women's Hospital. His clinical interest is in rheumatoid arthritis and his current research focus is on the physiological and pathological roles of protein citrullination, particularly in the setting of autoimmune diseases. He has discovered that several major risk factors of rheumatoid arthritis are associated with local or systemic hypercitrullination and that hypercitrullination can modulate the function of several types of immune cells, including neutrophils, T cells, and B cells, thereby contributing to the development of rheumatoid arthritis. His past and current research supports include funding from Rheumatology Research Foundation, National Health Research Institutes of Taiwan, Department of Defense, and National Institutes of Health.

Anna Huttenlocher

University of Wisonsin, US

Citrullination in zebrafish wound responses

Motile cells navigate through complex tissues in vivo during tissue repair and regeneration. We exploit the optical transparency of zebrafish larvae to image cell migration during wound repair. We will discuss the heterotypic cell-cell interactions and extracellular cues that mediate interstitial cell migration and regeneration and the role of citrullination during repair.

Anna Huttenlocher studies the basic molecular mechanisms that regulate cell migration and is interested in the implications of these mechanisms to human disease. Her laboratory has developed approaches to visualize and manipulate cell motility and innate immunity in zebrafish.

Mariana Kaplan

National Institutes of Health, US

Citrullination regulates autoimmunity

The early events leading to the development of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) remain unclear. In RA, formation of autoantibodies to citrullinated protein antigens (ACPAs) is considered a key pathogenic event. Neutrophils isolated from patients with various autoimmune diseases display enhanced neutrophil extracellular trap (NET) formation, a phenomenon that exposes autoantigens in the context of immunostimulatory molecules and that is dependent on the function of PADs. This presentation will highlight mechanisms by which citrullination of autoantigens and NETs contribute to the pathogenesis of a number of systemic autoimmunity and autoinflammatory syndromes, and potential therapeutic targets. Other posttranslational modifications will also be discussed.

Mariana Kaplan, M.D. is a NIH Distinguished Investigator, Chief of the Systemic Autoimmunity Branch and Deputy Scientific Director at NIAMS/ National Institutes of Health in Bethesda, United States. Dr. Kaplan is a rheumatologist and physician scientist whose lab studies mechanisms by which the innate immune system is involved in the initiation and perpetuation of systemic autoimmune diseases. She has published over 235 peer reviewed publications and has served in numerous roles at the American College of Rheumatology, the American Association of Immunologists, and the Lupus Foundation of America. She is a member of the American Society for Clinical Investigation, Association of American Physicians and the National Academy of Medicine. She has received the Henry Kunkel Young Investigator Award and the Edmund L. Dubois Memorial Lectureship, both from the American College of Rheumatology the 2015 Evelyn V. Hess Award from the Lupus Foundation of America in recognition of her significant contributions to lupus research, diagnosis, and treatment; the Charles L. Christian Award for significant impact on the understanding of lupus. She served on the Editorial Board of the Journal of Clinical Investigation and is Deputy Editor of Arthritis & Rheumatology.

Xisong Ke

Shanghai University of Traditional Chinese Medicine, CN

Chemical proteomics reveals small molecules targeting protein citrullination

Citrullination has been associated with various types of diseases including cancer and autoimmune disorders, whereas the therapeutic agents modulating citrullination is not available for clinical use so far. Recently we have initiated a collaborative project of target identification of traditional Chinese medicine, and globally profiled the endogenous binding proteins of 200 natural compounds from 90 research groups using quantitative chemical-proteasome approaches. Totally we have identified more than 1000 candidate binding proteins of these natural products in 70 cell models with a diversity of tissue origins. Most of the candidates are potentially involved to the established phenotypes of the natural products that were reported by the collaborative research groups. Of note, three natural compounds were found targeting peptidyl arginine deiminases (PADIs) mediating protein citrullination. The direct binding and function modulation of these PADIs and natural products were confirmed by various methods in vitro and in vivo. Our findings provide a unique opportunity to discover lead compounds modulating endogenous PADIs for disease therapy.

Xisong Ke is a Professor and the director in the Center for Chemical Biology, Shanghai University of Traditional Chinese Medicine, Shanghai, China. He obtained his PhD degree in Biochemistry from Peking Union Medical College (PUMC) in 2005. After a three years Postdoc training from 2006 to 2009 in University of Bergen (UiB), Norway, he received a Bergen Medical Research Foundation (BMSF) Researcher position in Department of Clinical Science of UiB from 2010 to 2016. At the end of 2016, he moved to Shanghai University of Traditional Chinese Medicine, where his research interests include small molecule targeting protein translational modification (neddylation, ubiquitination and citrullination), discovery and development of natural products for targeted cancer therapy, as well as investigation of understudied proteins using pharmacological approaches. He also serves a consulting editor of pharmacological Research since 2019.

Sigrun Lange
University of Westminster, London, UK

PADI-mediated modulation of extracellular vesicle signatures

PADI mediated post-translational deimination/citrullination causes structural and functional changes in a wide range of target proteins; facilitating protein moonlighting, modifying protein-protein interaction, causing neo-epitope generation and epigenetic changes. PADs also regulate biogenesis of extracellular vesicles (EVs), which are key players in cellular communication, including through transfer of cargo, e.g., proteins, non-coding RNAs and genetic material. PADs and EVs are linked to many pathobiological processes, including in central nervous system injury, cancers, and infection. Pharmacological PAD inhibitors have shown great promise in several in vitro and in vivo CNS injury models, cancers, autoinflammatory disease and host-pathogen interactions. As circulating EVs can be used as non-invasive liquid biopsies, their specific cargo-signatures may allow for disease "fingerprinting" and aid early diagnosis and response to therapy. PAD homologues are also found in bacteria and parasites, and PAD inhibition has shown changes in EV release leading to increased sensitivity to antibiotics and reduced attachment to host cells. This talk discusses PADI-mediated modulation of EV signatures in pathobiology.

Prof Sigrun Lange has a BSc in Biology, an MSc and a PhD from the Faculty of Medicine, University of Iceland. She held 3 post-doctoral research posts at University College London in neuroscience and regenerative medicine, pioneering discoveries of PADs in CNS regeneration. As Professor of Molecular Pathobiology at the University of Westminster, Prof Lange leads a cross-disciplinary research programme in Experimental Pathology with a focus on PADs and EVs, elucidating novel mechanisms underpinning evolution of the immune system and fundamental pathobiological and physiological processes. Prof Lange uses in vitro human, in silico and in vivo comparative animal-models across the phylogeny tree, including in animal species with unusual immune and metabolic features such as resistance to cancer, ageing and hypoxia, as well as various models for neurodegenerative disease, tissue regeneration, and models of host-pathogen interactions and infection, for the discovery of novel mechanisms underlying key concepts in the evolution of immune responses and to inform human pathologies, for therapeutic and drug-directed strategies, for disease fingerprinting and biomarker discovery in human and animal health. Prof Lange fosters strong collaborations with cross-disciplinary academic, clinical and industrial partners in the UK, USA, Canada, South America and Europe.

Nicholas LaThangue

Oxford University, UK

Citrullination in inflammatory disease and cancer

Increasing evidence suggests that citrullination is a key process involved in inflammatory disease and cancer. Our aim is to understand the role of citrullination by identifying key targets and decipher the role in the disease context. In previous studies we identified the master transcription factor E2F to be under citrullination control, and further clarified its impact on other post-translational modifications including methylation and acetylation. In recent studies, we have found that RNA splicing is influenced by PADs through the citrullination of RNA binding proteins and splicing factors, with important differences apparent in diseased compared to normal cells. Our results suggest that PADs have widespread effects on gene expression mediated at multiple levels. The evolving information on their biological roles has in turn fuelled significant interest in exploiting PADs as a therapeutic target in cancer.

Nick La Thangue is Professor of Cancer Biology at Oxford University. He is a Fellow of the Royal Society of Edinburgh, the Academy of Medical Sciences, the European Academy of Cancer Science, the Lister Institute and Professorial Fellow of Linacre College Oxford. He has over 200 publications and is an inventor on numerous patents. He has founded several companies focused on developing innovative therapeutics for cancer.

Kimberly Martinod

Department of Cardiovascular Sciences, KU Leuven, BE

PADI4 function in Immunothrombosis

Dr. Martinod will discuss the contribution of neutrophil extracellular traps (NETs) to thromboinflammation in chronic settings. Citrullinated histones, generated during NET formation by peptidylarginine deiminase 4 (PAD4) serve as useful biomarkers of ongoing NET formation. Studies in animal models show a protective effect of PAD4-deficiency in conditions of cardiac fibrosis and deep vein thrombosis. Cancer and metabolic syndrome both provide environments amenable to excess NET release in circulation, promoting pathological consequences systemically including in thrombosis and in cardiac dysfunction. PAD4 contribution to extracellular trap formation in other cell types, including eosinophils, will be discussed.

Kimberly Martinod is an assistant professor in the Department of Cardiovascular Sciences, Faculty of Medicine, at KU Leuven. Her research group, located at the Center for Molecular and Vascular Biology in Leuven, Belgium, focuses on the interplay between thrombosis and inflammation in cardiovascular disease with a focus on neutrophil extracellular traps.

Avinash Padhi
Karolinska Institutet, SE

PADIs in skin inflammation

An impaired skin barrier can trigger skin inflammation, which in turn can weaken the barrier even further, creating a vicious cycle. However, the specific mechanisms involved in this cycle are not yet fully understood. One of them is protein citrullination, that is conversion of peptidyl-arginine into peptidyl-citrulline by peptidylarginine deiminases (PADs). Even though citrullination is critical for skin homeostasis, the precise role of PADs in skin diseases remains unclear. Our studies show that citrullination is dysregulated during skin inflammation primarily through decrease in levels of PAD1 isotype. This decrease is limited to areas of the skin affected by inflammation. Among the targets of PADs in skin, citrullination of filaggrin is crucial for maintaining the skin barrier. A linear association between PAD1 levels and filaggrin citrullination suggested that reduced filaggrin citrullination in inflamed skin may be linked to decreased PAD1 levels. When exposed to cytokines associated with inflammation such as IL-22, IL-4, and IL-13, PAD1 levels decreased in skin cells. Importantly, this negative effect could be reversed using drugs commonly used to treat skin conditions, like Vitamin D, Acitretin, and Baricitinib. Together these findings could pave the way for application of PAD inducers as alternate therapy in inflammatory skin diseases.

Avinash Padhi completed his PhD in Biotechnology at KIIT University in India. During his doctoral studies, he focused on infection biology and innate immunity, specifically examining the host-pathogen interaction during Mycobacterial tuberculosis infection. Following this, he carried out his first post-doctoral research in the Dermatology division at Karolinska Institutet in Sweden. His work centered around immune responses in inflammatory skin diseases and their impact on the functioning of the skin barrier. Additionally, he has been investigating how infection influences immune responses in skin inflammation. Currently, Avinash is pursuing his second post-doctoral research in the Immunology & Allergy division at Karolinska Institutet, where he is studying mucosal CD4+T cell and B cell responses to Aspergillus antigens in patients with pulmonary sarcoidosis.

Ger Pruijn
Radboud University, NL

Rapid detection of anti-citrullinated protein antibodies in autoimmune patients

Anti-citrullinated protein antibodies (ACPA) are the most specific serological marker of rheumatoid arthritis (RA). Several ACPA-detection assays are available for clinical use, which are almost all based on ELISA(-like) assays with citrullinated peptides (CCP2). To facilitate ACPA-detection in low-volume laboratories and resource-poor environments, we aimed to develop a rapid and easy to perform test. An agglutination mediator was generated by protein engineering. Addition of this mediator to (diluted) blood samples results in hemagglutination when ACPA are present, which can be detected by the naked eye. A single-chain antibody fragment that binds to glycophorin A, one of the major surface proteins of erythrocytes was conjugated to an ACPA-binding citrullinated peptide. The applicability was assessed by the analysis of fresh blood samples from 200 RA patients and from 100 psoriatic arthritis (PsA) patients as a control group. The addition of the mediator resulted in detectable agglutination in 48-61% of the RA samples. Agglutination correlated well with the results obtained with a commercial anti-CCP2 ELISA (63-67%). Efficient agglutination was observed with only 9% of the PsA samples. We conclude that the agglutination mediator allows the rapid and efficient detection of ACPA by hemagglutination in human blood samples.

Ger Pruijn received his PhD degree in Physiological Chemistry from the University of Utrecht (1989). His interest in autoimmunity originates from his postdoctoral work at the University of Nijmegen, which was awarded with a fellowship from the Royal Netherlands Academy of Arts and Sciences. He has a special interest in autoantibody-based diagnostics and autoantigenic macromolecular complexes. After assistant and associate professorships in Biochemistry, he was appointed as full professor in Biomolecular Chemistry at the Radboud University in 2006. Since 2006 he is heading the Biomolecular Chemistry department and since 2021 also of the Biophysical Chemistry department of the Institute for Molecules and Materials. From 2015 till 2019 he was director of the Educational Institute of Molecular Sciences (Radboud University). He has (co-)authored more than 300 scientific papers, is co-inventor of 3 patents, co-founder of the companies ModiQuest and Novio Catalpa, and acted as consultant to several diagnostic companies.

Marco Radic

University of Tennessee Health Science Center, US

Shifting neutrophils into gear, the role of cytoskeletal citrullination

Neutrophils respond to inflammation with adhesion to endothelia, extravasation into tissues, and migration toward the site of infection or injury. Migration is dependent on the interplay of various adhesion molecules, yet integrin αMβ2 play a dominant role in guiding neutrophils toward greater concentrations of chemoattractants. The precise and timely neutrophil response to a localized infection is crucial in the early phase of an immune response. Redundant mechanisms exist to ensure directed migration toward and capture of microbial pathogens. We have shown that one of the crucial responses to inflammation drives activation of peptidylarginine deiminase (PAD4) and the release of neutrophil extracellular traps (NETs). Additional contributions to the response to inflammation include the deimination of cytoskeletal substrates. These include various actin cytoskeletal regulating proteins, including profilin, drebrin-like protein, and coronin 1A. Here, we examine ezrin, which along with radixin and moesin form the ERM complex of proteins that act as cytoskeletal adaptors. The ERMs bind to cytoplasmic tails of transmembrane proteins, including Mac-1 integrins, and link them to actin filaments. Phosphatases and kinases regulate the transition between the active and inactive conformations of ERMs. Here, we examine ezrin deimination and its role in neutrophil adhesion during neutrophil swarming and directed migration.

Dr. Radic received his Bachelor's in Science in Genetics from the University of California in Davis and his Ph.D. in Molecular Biology from the University of California in Irvine. He trained as Postdoctoral Researcher at the Fox Chase Cancer Center in Philadelphia. He started his independent lab research at the Medical College of Pennsylvania in 1991 and he moved to the University of Tennessee in 2000.
Research in the Radic focuses on basic mechanisms of autoimmunity and development of cell therapies for lupus. The Radic lab seeks to examine how autoantigens are externalized during cell death and engage with receptors on B cells. Radic and colleagues observed how neutrophil extracellular traps (NETs) provide a direct molecular link between inflammation and the induction of autoantibodies, a crucial step toward self-sustaining autoimmunity. The implications of these studies are that one critical nexus in the development of lupus lies in the sustained activation of autoreactive B cells. To decisively attack these B cells, the Radic lab applied immunotherapy with anti-CD19 CAR-modified CD8+ T cells in two murine models of lupus. The results of the initial studies encouraged first in human trials of CAR T cells in SLE. Dr. Radic is the recipient of numerous awards and honors, including the 2024 Lupus Insight Prize.

Guy Serre

Toulouse Institute for Infectious and Inflammatory Diseases, FR

Flying over 40 years of research on anti-citrullinated protein antibodies: diagnosis, pathophysiology and specific immunotherapy of Rheumatoid Arthritis.

Antibodies associated to Rheumatoid Arthritis (RA) had been described by indirect immunofluorescence on epithelial tissues. We confirmed their high diagnosis specificity and identified their targets as deiminated forms of (pro)filaggrin then as deiminated fibrin abundant in the inflammatory synovial tissue of patients. Those results led to immunoassays allowing detection of IgG anti-citrullinated protein autoantibodies (ACPA) that, after years of international validation, were recognized as a major diagnosis criterion for RA.

We showed that ACPA are secreted by plasma cells in the synovium and we proposed that formation of immobilized immunecomplexes on citrullinated fibrin deposits chronically generated in the inflammatory tisue by fibrinogen extravasation, polymerization and deimination by local PAD2 and PAD4 enzymes, is responsible for inducing and maintaining synovium inflammation. Using an in vitro model with human fibrinogen and macrophages, we demonstrated that ACPA do induce secretion of TNF and other proinflammatory cytokines, secretion being greatly amplified in the presence of IgM Rheumatoid Factor by forming macro-immunecomplexes.

Based on this pathopysiological model and epitopic mapping of citrullinated fibrin, we created the « Cure-RA » consortium to develop a bioimmunotherapy aiming to kill the ACPA B cells in the patients. We designed hybrids including peptides presenting immunodominant epitopes and either human Fc fragments of IgG or Fab fragments of monoclonals to plasma cell membrane antigens, to specifically target either the ACPA-positive B cells or the ACPA-secreting plasma cells, respectively.

Guy SERRE, emeritus Professor at the Université Toulouse 3, is working at the INFINITY Institute.
MD, PhD and Pathologist, Professor of Cell Biology at the Faculty of Medicine and Head of a laboratory at the University-Hospital, he founded and led for 25 years the INSERM-CNRS research laboratory « Epidermis Differentiation and Rheumatoid Autoimmunity ».
He participated to the discovery of several human epidermis proteins, the description of their structure, functions and pathophysiological involvement in various human diseases including genodermatoses.
His group identified the target proteins of autoantibodies associated to Rheumatoid Arthritis (RA) as deiminated forms of (pro)filaggrin then deiminated fibrin. That led to immunoassays for detection of anti-citrullinated protein antibodies (ACPA), now recognized as a major diagnosis criterion for RA. The group also demonstrated the pathophysiological involvment of immunecomplexes formed in the joint by ACPA and Rheumatoid Factor.
Now he leads a research consortium aiming at development of new specific immunotherapies for RA

Priyanka Sharma

Institute of Pharmacology and Structural Biology, FR

Citrullination dictates the transcriptional event

Eukaryotic transcription regulation is crucial for establishing how cells respond to various signals and maintain proper gene expression patterns. Post-translational modifications (PTMs) play an essential role in modulating the activity of the transcriptional machinery and chromatin structure. To fully understand cell functioning and the molecular basis of pathological conditions, it is necessary to understand the molecular principles that enable PTMs to modulate transcriptional regulation. Protein citrullination (deimination) is the PTM of arginine to the non-coded amino acid citrulline, catalyzed by the peptidyl arginine deiminases (PADIs) enzyme family. We previously showed that PADI2 specifically citrullinates the RNAPII to dictate the active transcription required to maintain cell proliferation. Functionally, Cit1810 enhances the association of active RNAP2 with the positive transcription elongation factor-b kinase complex. In this way, Cit1810 promotes the transcription regulation of essential genes contributing to cell identity.
Our finding provides a new spectrum to the functional implication of citrullination to the transcription machinery.
I will discuss our unpublished data on the potential role of PADI2-mediated citrullination on transcriptional events.

Priyanka Sharma is a principal investigator of the Epigenetic mechanism in the cancer team at the Institute of Pharmacology and Structural Biology (IPBS), Toulouse, France. She completed her Ph.D. in biomedical science in India by studying DNA methylation in coronary artery diseases. During her postdoctoral training at the Institute Pasteur, Paris, France, and the Center for Genomic Regulation, Barcelona, Spain. She focused on chromatin biology by exploring the role of citrullination. She started as an independent group as an INSERM fellow at IPBS in 2023.
She is committed to bringing the citrullination community together to the forefront.

Miriam Shelef

University of Wisconsin-Madison, US

PADIs and Citrullination in Extracellular Traps, Autoantibodies, and Rheumatoid Arthritis

Citrullination has been a major focus among investigators studying the pathophysiology of rheumatoid arthritis (an autoimmune, inflammatory arthritis), given the high specificity of anti-citrullinated protein antibodies (ACPAs) for this disease.
This talk will discuss findings related to neutrophil and macrophage extracellular traps as potential sources of citrullinated antigens bound by ACPAs, the role of citrulline-containing IgG epitopes as unifying antigens for the two major autoantibody types in rheumatoid arthritis (ACPAs and rheumatoid factors), and the contributions of two citrullinating enzymes, peptidylarginine deiminase 2 and 4, in murine models of rheumatoid arthritis.

Miriam Shelef is an Associate Professor of Medicine at the University of Wisconsin School of Medicine and Public Health in Madison, Wisconsin, USA. She completed her MD and PhD at Columbia University and her internal medicine residency and rheumatology fellowship at the University of Wisconsin. Her research has applied murine models, clinical samples, high density peptide array, and novel statistical methods to the study of rheumatoid arthritis and, more recently, COVID-19, leading to the discovery of important roles for the citrullinating peptidylarginine deiminases in inflammation and inflammatory arthritis as well as identifying key features of the antibody and autoantibody repertoire in rheumatoid arthritis and COVID-19. Her work continues to focus on the pathophysiology of rheumatoid arthritis as well as the intersection between a normal and abnormal immune response.

Michel Simon

Toulouse Institute for Infectious and Inflammatory Diseases, FR

Peptidylarginine deiminases and deiminated proteins at the epidermal barrier

The epidermis is a barrier for preventing penetration into the skin of pathogens, allergens, toxins and UV radiation. It also avoids the uncontrolled loss of body solutes. Finally, it acts as a mechanical protection. For many years, we are interested in the role of peptidylarginine deiminases (PADs) in the epidermis. Three PADs are expressed in the epidermis, PAD1-3. Their expression/activity is regulated at the transcriptional, translational and posttranslational levels. Whereas the specific role of PAD2 remains enigmatic, PAD1 and PAD3 are involved in the epidermal barrier function, keratins, LL37 anti-microbial peptide, filaggrin and filaggrin-related proteins being the most abundant deiminated epidermal proteins. Deimination promotes the cross-linking of most of them to the cornified envelopes. Furthermore, citrullination of filaggrin is a limiting step in filaggrinolysis, which leads to the production of amino acids and amino acid derivatives essential for proper upper epidermis hydration and plasticity, desquamation, UV protection and epidermal barrier homeostasis. Filaggrinolysis allows the adaptation of keratinocytes to a dry external environment. PAD1 is a regulator of the autophagy process associated with the ultimate step in keratinocyte differentiation. Finally, dysregulation of epidermal citrullination may be involved in the pathogenesis of inflammatory skin diseases, including psoriasis, atopic dermatitis and hidradenitis suppurativa.

Dr Michel Simon got his PhD degree from the University of Geneva, Switzerland, in 1988 and subsequently moved to the Department of Ontogenesis and Molecular Genetics of Laval University in Quebec, Canada. In 1990, he joined the Toulouse University, France. He was recruited as a tenured scientist by the National Institute for Health and Medical Research (INSERM) in 1998. He currently heads a team in the Toulouse Institute for Infectious and Inflammatory Diseases (INFINITy, Inserm U1291 - CNRS U5051 - Toulouse University). He is interested for 20 years in the characterization of keratinocyte terminal differentiation and in the importance of the epidermal barrier function in skin homeostasis and skin inflammatory diseases. In particular, he was one of the pioneers in the study of citrullination in human skin. He published more than 110 publications referenced in Web of Science, as well as several book chapters and patents.

Paul Thompson

UMass Chan Medical School, US

Functional role of citrullination in human diseases

The Protein Arginine Deiminases (PADs) hydrolyze arginine residues to form citrulline. This post-translational modification, termed citrullination, is upregulated in several autoimmune disorders including Rheumatoid Arthritis (RA), lupus, and Alzheimer's disease. While these enzymes are important regulators of gene transcription, the full spectrum of biological activities is relatively underexplored. Herein, I will discuss our development of pan and isozyme specific PAD inhibitors and their use *in vitro* and *in vivo*.

I will specifically highlight our efforts to generate next generation panPAD inhibitors as well as PAD2 selective inhibitors for the treatment of sepsis and other inflammatory diseases that are associated with increased levels of PAD2 activity.

Paul Thompson is the Director of the Program in Chemical Biology at the University of Massachusetts Medical School. He is also a Professor in the Department of Biochemistry and Molecular Biotechnology. Paul is a world leader in the biology and biochemistry of the Protein Arginine Deiminases, a family of enzymes whose activity is increased in rheumatoid arthritis, inflammatory bowel disease, lupus, and cancer. In 2014, Paul founded Padlock Therapeutics to develop therapeutics that target these enzymes. This company was subsequently acquired by Bristol Myers Squibb and a PAD inhibitor is now in clinical trials.
Paul received his BSc and PhD degrees from McMaster University and then performed postdoctoral research at Johns Hopkins. Paul began his independent career at the University of South Carolina before moving to Scripps Florida. Paul subsequently moved to the University of Massachusetts Medical School in Aug 2014. Paul has published more than 200 articles and is a Fellow of the Royal Society of Chemistry.

Louise Walport

The Francis Crick Institute, UK
Imperial College, UK

Exploring PADI regulation using cyclic peptide tools

Peptidyl arginine deiminases are implicated in a wide range of patho(physiological) processes including the innate immune response, skin homeostasis and early embryonic development. Much of this activity is attributed to catalytic citrullination of arginine residues in diverse substrate proteins including histone proteins, filaggrin and myelin basic protein. Despite their wide-ranging biological roles, the mechanisms through which PADIs are regulated within cells remain poorly understood. Combining cyclic peptide tool development with biochemical and structural approaches we have been exploring the mechanisms of PADI activation. In this talk I will present two stories of recent work from our lab: Firstly, I will present our work to develop cyclic peptide activators of PADI4. Our lead peptide activates citrullination both in vitro and in cells.
CryoEM studies reveal that activation occurs through binding to the allosteric hinge region of PADI4, providing support for a similar regulatory mechanism in cells by which PADI4 could be activated by allosteric protein binding partners. Secondly, I will present recent structural work from our lab exploring the non-catalytic role of PADI6 in early embryonic development, shedding light on the elusive final member of the PADI family, PADI6.

Louise Walport is a Senior Lecturer in the Department of Chemistry at Imperial College London and a Group Leader at the Francis Crick Institute. She obtained her doctorate from the University of Oxford in 2014 under the supervision of Prof. Chris Schofield and Prof. Christina Redfield, focussing on mechanistic studies of histone demethylases. Following further postdoctoral work in Oxford, she was awarded a Marie Skłodowska-Curie Global Fellowship to work in the group of Prof. Hiroaki Suga at the University of Tokyo, where she developed her interest in cyclic peptides. She established her independent group in late 2018, where she continues to be interested in understanding enzyme-catalysed post-translational modifications, in particular protein citrullination, and developing new approaches to probe these with cyclic peptide-based tools.

Zuoshang Xu

University of Massachusetts Chan Medical School, US

Protein Citrullination in Amyotrophic Lateral Sclerosis

Abnormal protein citrullination (PC) and dysregulation of peptidyl arginine deiminases (PADs) are associated with numerous pathological conditions, including inflammatory diseases and neurodegeneration. Inhibition of PADs in animal disease models has shown therapeutic efficacy, suggesting a role of PC in pathogenesis. To determine whether PC contribute to amyotrophic lateral sclerosis (ALS), a deadly neurodegenerative disease characterized by loss of motor neurons, paralysis, and eventual death, we investigated PC in two transgenic mouse models of ALS. Both models show that PC and PAD2 increase progressively in reactive astrocytes, while decreasing in neurons. Importantly, PC accumulates progressively in protein aggregates that contain the myelin proteins PLP and MBP during disease progression. Finally, increased PC and PAD2 expression spatially correlate with areas of the CNS with the most severe motor neuron degeneration. These findings are replicated in human ALS tissues. Ongoing and future studies are focused on the impact of PAD2 knockout in ALS mouse models and mass spectrometry analysis of citrullinated proteins. Our results suggest that altered PC is an integral part of the neurodegenerative process and potential biomarkers for disease progression in ALS. Moreover, increased PC may contribute to disease-associated processes such as myelin protein aggregation, myelin degeneration, and astrogliosis.

Zuoshang Xu is a Professor of Biochemistry and Molecular Biotechnology at the University of Massachusetts Chan Medical School. His lab studies ALS, a devastating and fatal neurodegenerative disease. His work aims to understand the disease mechanism and develop therapy. His lab has contributed to defining the roles of mitochondrial damage and loss and gain of TDP-43 function in ALS and constructing several ALS transgenic mouse models. He has also helped develop gene silencing strategies to treat ALS. Recently, his lab teamed up with Paul Thompson's lab to investigate protein citrullination (PC) in ALS. The work has unveiled dramatically altered PC and PAD2 expression during ALS progression (Yusuf et al. 2022, PMID: 36076282; 2024, PMID: 38253209). They are currently defining the ALS citrullinome and PC's impact on the disease. Their future work will focus on developing PC as new ALS biomarkers and testing treatment of the disease by inhibition of PC.

Li Yang

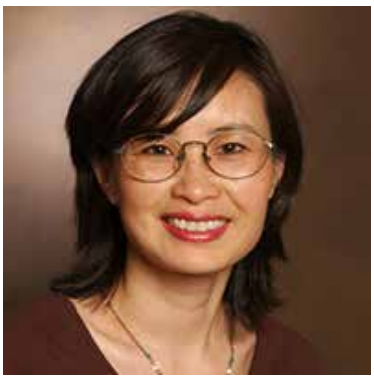
Center for Cancer Research, National Cancer Institute, NIH, US

Histone 3 (H3) citrullination leads to nuclear expulsion of apoptotic cancer cells and enhanced metastatic outgrowth.

Protein citrullination catalyzed by Peptidylarginine deiminase (PADI) is a critical regulatory mechanism in physiology and pathology conditions. In cancer, PADI4 is highly expressed in metastatic tumor cells and citrullinates histone H3, leading to loss of the positive charges and a rapid chromatin decompaction or expansion and subsequent nuclear bursting or expulsion. The resulting extracellular DNA-protein complex is enriched in ligands for receptor for advanced glycation endproducts (RAGE). The chromatin-bound RAGE ligand S100a4 activates RAGE receptors in neighboring surviving tumor cells, leading to Erk activation and metastatic outgrowth.

Most tumor cells undergo apoptosis in circulation and at the metastatic organ sites due to host immune surveillance as well as therapeutic treatment.
Tumor cells are also highly heterogeneous, exhibiting diverse responses when exposed to different environments. Our studies reveal a mechanism of tumor heterogeneity in apoptosis by which some tumor cells die for the benefits of the population's survival through Padi4-mediated nuclear expulsion.

This talk is supported by Toulouse Cancer Fundation



Dr. Li Yang is a Senior Investigator at the National Cancer Institute. She received her Ph.D. in the Department of Cancer Biology at Vanderbilt University, under the mentorship of Dr. David Carbone. Her dissertation research focused on COX-2 pathway, immune suppression, and the contribution of host myeloid cells to tumor progression. She investigated TGF-β regulation of inflammation and tumor microenvironment during her postdoc research with Dr. Harold Moses. She joined NCI as principal investigator in 2009 and was tenured in 2016.
Dr. Yang has contributed to the identification of the immune and inflammatory mediators in the anti- and pro-tumor functional switch of TGF-β, as well as myeloid TGF-β signaling in cancer immune surveillance and in inflammatory stroke. Her laboratory recently discovered a previously unreported mechanism of tumor heterogeneity in apoptosis, through which some tumor cells die for the benefits of the population's survival through Padi4-mediated nuclear expulsion.

Xuesen Zhang
Nanjing Medical University, CN

The novel role of PADI2 in regulating ERs signaling in breast cancer cells

We previously have demonstrated that, in estrogen receptor positive (ER+) breast cancer cells, the nuclear PADI2 targets histone H3R26 for citrullination and specifically facilitates ER binding by modifying nucleosome structure. However, the role of cytoplasmic PADI2 (major form PADI2 in cells) in estrogen signaling is still unclear.
There are two ER subtypes, ERα and ERβ, that belong to the nuclear receptor family, once activated by estrogen, have different biological functions as they may regulate different targets.
ERα is frequently detected in the early development of breast cancer tissues and is associated with a poor prognosis and an increased proliferation rate, while the presence of ERβ indicates better survival and is generally regarded as an anti-tumor factor. Changes in the ratio of ERα/ERβ protein levels determine the balance between cell proliferation and apoptosis. Here, we will discuss the novel role of cytoplasmic PADI2 in regulation of ratio of ERα/ERβ protein levels in breast cancer cells. Eventually, PADI2 inhibitors combined with endocrine therapy drugs for breast cancer may help retard or prevent the occurrence of endocrine therapy resistance for ER+ breast cancers.

Dr. Xuesen Zhang, a Professor in the Department of Histology and Embryology at China Medical University, China. He received Bachelor's degree and Master's degree from Dalian Medical University, China, in 1999 and 2002, respectively. Upon completion of his doctorate in Chinese Academy of Sciences in 2005, Dr. Zhang accepted his postdoctoral training at the University of Iowa, USA. In 2007, he joined Prof. Scott Coonrod Lab at Cornell University, USA and received more comprehensive postdoctoral training in the study of citrullination and breast cancer. In 2013, he was appointed as a Principal Investigator in Nanjing Medical University. In 2022, he moved to China Medical University and was appointed as Dean of Department of Histology and Embryology. Dr. Zhang's research has mainly focused on the role of non-histone protein citrullination in regulating tumor cell signaling pathways. He was awarded Susan G. Komen for the Cure postdoctoral fellowship in 2010, and the Distinguished Professor of Jiangsu Province, China in 2016.

Selected talks

PADI inhibition overcomes the acquisition of resistance after KRAS blockage in pancreatic ductal adenocarcinoma cells

Francesca Agostini , Annalisa Ferino , Ylenia Cortolezzis , Valentina Rapozzi , Luigi E. Xodo , Eros Di Giorgio

Department of Medicine, University of Udine, Italy

In pancreatic ductal adenocarcinoma (PDAC) KRAS activating point mutations are frequently observed. KRAS inhibitors specific for the G12C mutation have been developed to selectively target cancer cells. However, KRASG12C cells display metabolic adaptations and rapidly acquire resistance to the treatment. Peptidyl Arginine Deiminases (PADIs) catalyze the conversion of peptidyl-arginine into citrulline residues, influencing the activity of targeted enzymes and modifying the state of the chromatin by histone citrullination. Although there is evidence that PADIs can modulate the activity of enzymes involved in metabolism, the role of these proteins in sustaining cancer cells remains to be clarified. In this project we aim to evaluate the contribution of PADIs in controlling the metabolic resetting of cancer cells that have acquired resistance to KRAS inhibition.
We studied the PDAC cell line MIA PaCa-2 and evaluated the effect of KRASG12C degradation mediated by the PROTAC LC-2 in combination with PADI inhibition obtained by the pan-PADI inhibitor BB-Cl-amidine. Colony formation assay and biochemical assays were used to evaluate the viability of cancer cells and the metabolic resetting achieved after co-treatment. In MIA PaCa-2 cells, KRASG12C forced degradation led to PADI1 and PADI3 down-regulation. Conversely, MIA PaCa-2 cells developing LC-2 resistance increased PADI1 and PADI3 protein levels. Interestingly, PADI inhibition with BB-Cl-amidine had a limited effect on viability of MIA PaCa-2 cells if used alone, but displayed a strong synergic anti-neoplastic effect when combined with LC-2 treatment. Indeed, the co-treatment with LC-2 and BB-Cl-amidine rapidly induced apoptosis in PDAC cells, leading to a drastic decrease of ATP level and colony formation. These data suggest that PADIs play a fundamental role in the metabolic adaptations observed in LC-2 resistant cells. Thus, a combined approach that targets KRASG12C and PADIs appears to be promising in PDAC therapy.

PADI4 regulates p53-mediated tumor suppression and post-translationally modifies p53 via citrullination

Alexandra Indeglia[1, 2], Andrea Valdespino[1, 3], Giulia Pantella[1, 4], Connor Hill[1], Maya Foster[1], Kaitlyn Casey[1], Hsin-Yao Tang[1], Alessandro Gardini[1], Maureen Murphy[1, 2, 3]

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TP53 is the most frequently mutated gene in human cancer. While it is well understood that the ability of p53 to act as a transcription factor is required for tumor suppression, the key target genes downstream of p53 required for tumor suppression are still incompletely understood. We identified the p53 target gene, PADI4, to be exquisitely sensitive to p53 mutation, and loss of PADI4 is seen in transcriptionally competent p53 hypomorphs. We have now found that PADI4 is the top gene showing impaired transactivation by three different p53 cancer associated variants. PADI4 is a regulator of histone modification and gene transcription via citrullination, which is the process of deiminating arginine to the non-natural amino acid citrulline. Our TCGA analysis reveals PADI4 is downregulated or mutated in multiple human cancers. Surprisingly, we show that PADI4 is sufficient to suppress tumor growth and sensitize wild-type p53 cells to chemotherapeutics. We further show that PADI4 is potently tumor suppressive in vivo, and this tumor suppression is dependent on an intact immune system. PADI4 enhances the transactivation of p53 targets and genes involved in immune activation. In addition, we identify a p53–PADI4 gene signature that is predictive of survival and the efficacy of immune-checkpoint inhibitors. We have further found that PADI4 interacts and modifies p53 via citrullination at key residues within the nuclear localization sequence and C-terminal domain. PADI4 colocalizes with p53 on chromatin, and redirects p53 to non-canonical target genes and potentially novel target genes. The findings from this study reveal PADI4 as not only a key target gene of p53, but a core regulator of p53 activity and target specificity through a novel protein modification. This work highlights the need to reassess the role of PADI4 in cancer, and also provides insight into critical downstream target genes important for tumor suppression by p53.

Quantifying Neutrophil Extracellular Trap Release and Citrullinated Histone H3 in an Infection-Inflammation NET-Array Microsystem

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Introduction: Excessive release of neutrophil extracellular traps (NETs) has been linked to various human pathologies, notably COVID-19, where elevated levels are indicative of increased risks of coagulopathy and immunothrombosis. Traditional immunoassays often lack single-cell resolution and struggle to capture the complexities of microenvironments. Human microphysiological models (microsystems) enable quantification of single-cell dynamics and behavioromes, such as NETosis, within physiologically relevant microenvironments.

Aims: Our objective was to develop a NET-array microsystem capable of accurately quantifying human citrullinated histone H3-positive NET-release at a single-cell level within infection and inflammation-rich microenvironments.

Methodology: The NET-array microsystem, featuring open chambers and constricted loops, was designed to simulate infection and inflammation-rich microenvironments. Primary human neutrophils were exposed to Pseudomonas aeruginosa PAO1 and inflammatory cytokines, including tumor necrosis factor-α and interleukin-6. Time-lapse imaging captured the release of NETs, including citrullinated histone H3-positive NETs, while computer-vision-based image processing methods were developed to automate quantification.

Main Results: Our study unveiled a significant increase in NET release in response to Pseudomonas aeruginosa PAO1 when combined with inflammatory cytokines compared to infection alone. Notably, we measured increased citrullinated histone H3-positive NET-release to PAO1 when challenged with tumor necrosis factor-α and interleukin-6, assessed at the conclusion of the live–dead NETs assay. Additionally, confinement within loops of the device led to reduced NET release. Our NET-array device is currently deployed at the University of Texas at Southwestern Medical Center for high-throughput screening of novel immunotherapies aimed at fine-tuning NET release to mitigate pathological neutrophil-driven inflammation.

Non-protein activators of PAD4

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Peptidylarginine deiminase 4 (PAD4) is citrullinating enzyme involvement in pathogenesis of rheumatoid arthritis. Activation of this enzyme by calcium remains unclear as calcium levels in the human body are typically too low for full activity. It has been proposed that allosteric activators of PAD4 improve calcium affinity of the enzyme. Several PAD4 activators have been identified recently, including optimized synthetic cyclic peptides or specific antibodies. They are results of iterative optimization or in vitro selection, not physiological molecules. Herein, we investigated the activation of PAD4 by glycosaminoglycans (GAGs) using heparin as model. We employed activity assays, chromatography, molecular interaction measurements (MST and SPR) and CryoEM to show activation of PAD4 and formation of PAD4-heparin complex.

Our data show that PAD4 binds heparin, even at high salt concentrations. PAD4 is activated in the presence of heparin at sub-optimal concentrations of calcium and we observe that the activation mechanism depends on increasing PAD4 calcium affinity.

We further demonstrate that activation by heparin depends on the length and charge of GAG molecule. Direct binding measurements using MST and SPR confirmed a tight interaction between PAD4 and heparin. CryoEM structures revealed PAD4 bound to heparin oligomers of varying lengths, showing different binding modes and supramolecular organizations of PAD4-heparin complexes. Mutagenesis studies indicated that PAD4 dimerization is essential for efficient activation. We demonstrate that cell surface binding of PAD4 depends on the GAG composition using CHO cell models and that heparin induces histone H3.1 citrullination and DNA release from human neutrophils. Additionally, other GAGs were identified as PAD4 activators. In summary, our findings present the first natural activators of PAD4, potentially explaining its role in physiological processes related to rheumatoid arthritis development.

Deep Learning Boosts Citrullination Identification in Mass Spectrometry-Based Proteomics

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Detecting protein citrullination remains challenging due to their low abundance and limited enrichment tools. Direct identification of citrullination sites by high-resolution mass spectrometry has offered biological insights when enrichment is unavailable. While modern mass spectrometers are sensitive and accurate, errors derived from database searching remain dominant, especially with the same mass increase of deamidation (NQ). Manual inspection of candidate spectra becomes crucial to validate citrullination identification but hinders throughput in large-scale studies. Here, we present a precise and sensitive data analysis workflow to identify citrullination sites in proteomics datasets.

This pipeline boosts identification by a deep learning model, Prosit-Cit, to predict the retention time and fragment ion intensities of citrullinated peptides. Prosit-Cit is the extension of Prosit trained by ~53,000 spectra derived from ~2,100 synthetic citrullinated peptides. Our workflow achieves high precision in identifying citrullination, evaluated with seven dilutions of 200 synthetic citrullinated peptides spiked into cellular tryptic digests. Re-analyzing ten human tissue proteomes using this workflow retrieved the most known sites and identified 5-10 times more citrullinated sites. Extending the search to the Arabidopsis tissue proteome dataset detected over 1,000 citrullination sites across 30 tissues, marking the first large-scale citrullination report in plants. Specifically, we found a higher citrullination level in flowers and its ubiquity across tissues, suggesting broader significance of citrullination in Arabidopsis. This work presents a precise and high-throughput workflow for large-scale citrullination identification, setting a benchmark as the first survey of protein citrullination in plants and enabling biological discoveries of protein citrullination in both new and existing proteomics datasets.

Citrullinating enzyme PADI4 and transcriptional repressor RING1B bind in cancer cells

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Polycomb groups (PcGs) are transcriptional repressors, formed by a complex of several proteins, involved in multicellular development and cancer epigenetics. One of these proteins is the E3 ubiquitin-protein ligase RING1 (or RING1B), associated with the regulation of transcriptional repression and responsible for monoubiquitylation of the histone H2A. On the other hand, PADI4 is one of the human isoforms of a family of enzymes implicated in the conversion of arginine to citrulline; it is also involved in the development of glioblastoma, among other types of cancers. In this work, we showed the association of PADI4 and RING1B in the nucleus and cytosol in several cancer cell lines by using immunofluorescence and proximity ligation assays.
Furthermore, we demonstrated that binding was hampered in the presence of GSK484, an enzymatic PADI4 inhibitor, suggesting that RING1B could bind to the active site of PADI4, as confirmed by in silico experiments. In vitro and in silico studies showed that binding to PADI4 occurred for the isolated fragments corresponding to both the N-terminal (residues 1–221) and C-terminal (residues 228–336) regions of RING1B. Binding to PADI4 was also hampered by GSK484, as shown by isothermal titration calorimetry (ITC) experiments for the sole N-terminal region, and by both NMR and ITC for the C-terminal one. The dissociation constants between PADI4 and any of the two isolated RING1B fragments was in the low micromolar range (~ 2-10 microM), as measured by fluorescence and ITC. The interaction between RING1B and PADI4 might imply citrullination of the former, with several biological consequences, as well as a potential therapeutic relevance for improving cancer treatment with the generation of new antigens.

PAD4 controls tumor immunity via restraining MHC-II machinery in macrophages

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MHC-II–mediated antigen presentation is essential for the function of the immune system. Recent evidence indicates that MHC-II expression shapes tumor immunity and response to immunotherapy. MHC-II expressing antigen presenting cells (APCs) mediate CD4+ T-cell priming and activation and license them to support the CD8+ T-cell response. Citrullination – catalyzed by peptidyl arginine deiminases (PADs) – is a post-translational modification at arginine residues. Here, we show that PAD4 negatively regulates anti-tumor immunity by tuning IFNg-signaling gene expression, including MHC-II, in tumor associated macrophages (TAMs) in tumor bearing mouse models. Mechanistically, PAD4 citrullinates STAT1, thereby promoting the interaction between STAT1 and PIAS1 (protein inhibitor of activated STAT1) and resulting in reduced MHC-II expression in TAMs. Moreover, low levels of MHC-II expression in TAMs correlate with a reduced response to immunotherapy and are associated with poor cancer patient survival. The data suggest that targeting PAD4-mediated citrullination may serve as a potential anti-cancer therapeutic approach.

Cardiac PAD2 Expression and Myocardial Citrullination Decline with Age in Female Mice Independent of Estrogen

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Cardiac aging is sexually dimorphic, with women more likely than men to develop diastolic dysfunction (heart failure with preserved ejection fraction, HFpEF) for which no therapies exist. Loss of cardioprotective estrogen (E2) during menopause increases risk of HFpEF in women through unclear mechanisms. Expression of peptidylarginine deiminase 2 (PAD2) is positively regulated by E2, suggesting a potential novel mechanism linking PAD2, diastolic function, and E2 in the aging female heart. We hypothesized that PAD2 expression and protein citrullination decline with age in the female heart due to loss of E2, contributing to diastolic dysfunction. Global deletion of PAD2 exacerbated the HFpEF phenotype in aging females, with worse diastolic function than age matched controls. PAD2 expression and protein citrullination decreased with age in the female heart. Mass spectrometry detected citrullination of sarcomeric and metabolic proteins, with overall lower levels of citrullinated proteins in aged female hearts compared to young. To confirm direct regulation of PAD2 by E2, a cohort of young and aged mice underwent ovariectomy (OVX) with or without E2 replacement. Contrary to our hypothesis, no changes in PAD2 expression were observed in young females and PAD2 expression increased with OVX and OVX+E2 in aged females. Given the previous reports of hypoxia regulating PAD activity, we quantified expression of the transcriptional regulator hypoxia inducible factor-1α (HIF-1α). HIF-1α was upregulated by OVX and OVX+E2, suggesting a mechanism by which PAD2 is regulated by hypoxia. Together, we establish that protein citrullination and PAD2 in the female heart change with age, perhaps contributing to diastolic dysfunction. Elucidation of the mechanisms underlying PAD2 expression and function of citrullinated myocardial proteins remains to be determined and may benefit development of therapies for diastolic dysfunction in the aging female heart.

Citrullination of fibrinogen creates a pre-metastatic site to facilitate lung metastasis in cancer patients

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Tumor metastasis is one of the most challenging problems in cancer research. It is estimated that 90% of cancer-related deaths are due to metastasis, and the study of metastasis is needed. Tumor metastasis results from interactions between the primary tumor and secondary metastatic tissues. Primary tumor-derived molecular signals influence endothelial cells and immune cells in the distant organ to make the microenvironment pro-tumor. Once a circulating tumor cell reaches the microenvironment, the cell increases its chances of survival and extravasation into the tissue for tumor regrowth.
Because this microenvironment formation occurs before the tumor cells physically appear, it is referred to as the pre-metastatic region. In this study, we have demonstrated [Nature Commun (2023) 14, 4096] accumulation of citrullinated fibrinogens (citFbgs) in the pre-metastatic lung using our humanized serum amyloid A (SAA) mouse model and clinical specimens. Pulmonary endothelial cells mediate citrullination of fibrinogen and citFbgs form aggregation complexes that attract tumor cells. Our specific antibody to CitFbg clearly visualized the pre-metastatic sites in the lung, and this detection is the first step towards preventing metastasis.

Untangling a possible catalytic function of PADI6 in early embryo development

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Peptidyl arginine deiminase 6 (PADI6) is a maternal-effect gene essential for early embryo development and female fertility. PADI6 is the least well characterised of the PADIs - a family of enzymes that catalyse the post-translational conversion of peptidyl arginine to citrulline. Current literature suggests that the primary function of PADI6 in embryo development is structural, not catalytic, and PADI6 functions as a key structural component of the cytoplasmic lattices (CPLs). The CPLs are abundant structures, exclusive to the oocyte and early embryo, recently proposed to be a storage site for maternal proteins. Despite its clear biological and clinical significance, the exact function of PADI6 remains elusive. Crucially, there is currently no reported catalytic activity of PADI6.

In our work, we aimed to expand our understanding of the function of PADI6 in early embryo development, in particular, the question of whether PADI6 has a catalytic function. Using a pair of mouse models, we collected scRNA-seq data from oocytes and early embryos. Our data reveals that embryonic genome activation EGA is defective in embryos from Padi6 knock-out mothers, and also appears misregulated in embryos from mothers in which a mutation has been introduced into the PADI6 active site, despite their CPLs appearing intact. Together this suggests that there may be an additional non-essential function of PADI6 during EGA, independent of CPL formation. To further characterise these observations, we have established a single embryo proteomic workflow capable of consistently identifying upwards of 6000 proteins from single oocytes or embryos. Using this single embryo proteomic workflow, we have characterised the proteomes of GV oocytes, MII oocytes, zygotes and 2-cell embryos from each of our lines. These data further point towards an additional function of PADI6 in early embryo development, independent of the CPLs, which may involve catalysis.

Flash talks | Posters

Investigating PAD-mediated citrullination in HPV-driven head and neck squamous cell carcinoma

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Citrullination is a post-translational modification catalyzed by peptidyl-arginine deiminases (PADs), which convert peptidyl-arginine into peptidyl-citrulline. The PAD family in humans comprises five isozymes (PADs 1-4 and 6), which are implicated in various diseases, including cancer. Our previous findings revealed a novel mechanism by which high-risk human papillomaviruses (HPVs) manipulate host regulatory pathways involved in the cell cycle and survival to enhance viral fitness, implying that PADs may be promising targets for developing new host-targeting antivirals to prevent cervical cancer progression. In this study, we further explore the impact of PAD-mediated protein citrullination on HPV infection, in the context of the head and neck squamous cell carcinoma (HNSCC).

Here we demonstrate using RT-qPCR that PAD1, PAD2, and PAD4 are expressed and modulated in HNSCC biopsies compared to normal mucosa, though their regulation is not influenced by HPV status as tested by nested-PCR. Additionally, we conducted an immunohistochemical (IHC) analysis focusing on PAD1 and PAD4 in a cohort of formalin-fixed paraffin-embedded HNSCC samples.

According to preliminary results, PAD1 expression appears to be associated with tumor differentiation; specifically, we observed intense IHC positivity in poor differentiated HNSCC. Additional investigation is necessary for PAD4; however, its expression seems to overlap with PAD1 tumor expression. To further corroborate these data, we took advantage of an in vitro cellular model of immortalized keratinocytes (NOKs) expressing the major HPV oncoproteins E6 and E7. Preliminary results revealed that both PAD expression and overall citrullination are not significantly modulated by HPV oncoproteins. In conclusion, in the context of HNSCC, the expression of PAD1 and PAD4 is modulated according to tumor differentiation, although HPV infection does not appear to significantly influence this regulation.

Susceptibility of Complement system proteins on citrullination with human PAD4

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Protein citrullination and production of ACPAs are hallmarks of rheumatoidal arthritis (RA). Proteomic analysis of citrullinome of patients with RA revealed complement proteins as targets for PADs. The complement system (CS), essential element of the innate immunity is responsible for protection against microbes, effective clearance of necrotic and apoptotic cells, regulation of inflammation and control of the interplay between the innate and adaptive branches of immune system. The disruption of CS homeostasis leads to pathological processes like: destruction of self-cells and tissues in case of excessive activation, while impaired activation results in reduced efficiency of the clearance of the dead cells leading to the development of the autoimmune diseases (like RA).

The aim of our study was to investigate susceptibility of major CS proteins for deimination by PAD4 in vitro and to check how this modification influences their function. We confirmed efficient modification and mapped citrullination sites in major CS proteins using phenylglyoxal probes and MS. Citrullination of human serum showed decrease of total CS activity in both classical and alternative pathway, evaluated with hemolytic assays CH50 and AP50 respectively. Using CH50 assay and sera depleted for certain CS components we showed that functionally, among the most vulnerable CS proteins were C3, C4, C1q and C5, which lost around 50-70% of activity upon modification. Western Blot analysis of the activation process for C3 and C5 showed different effects of modification for those proteins, as citrullination decreased rate of C5 proteolytic activation, while decrease in C3 activity is related to impaired interaction of CitC3 with other proteins. Citrullination of major anaphylatoxins was confirmed with RP-HPLC and MS, showing citrullination of crucial C-terminal arginines. C5aCit showed impaired activity on U937-C5aR cells in calcium influx assay.

Inhibitors of peptidyl-arginine deiminases and histone citrullination decrease the angiogenic potential and Akt activation in human endothelial cells

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Citrullination or conversion of peptidyl-arginine to peptidyl-citrulline is PTM of proteins catalyzed by peptidyl-arginine deiminases (PAD). It alters the structure and function of proteins e.g. by modification of the active site. Increased levels of PAD and citrullinated proteins are linked to many pathological conditions, especially autoimmune disorders and cancer. The physiological role of this process is still poorly understood, particularly in endothelial cells (ECs), which regulate important processes including hemostasis and angiogenesis.This study aimed to determine how pharmacological inhibition of citrullination affects the functions of human vascular ECs.
Two models were used: an immortalized microvascular endothelial line (HMEC-1) and primary umbilical vein ECs (HUVEC). We used three irreversible PADs inhibitors: BB-Cl-amidine, Cl-amidine and F-amidine. To verify the research goal, we performed: a cytotoxicity test of the tested inhibitors; analysis of citrullination level of histone H3 (H3cit); assessment of the impact of PADs inhibition on the angiogenic potential (pseudocapillary structures formation, wound healing, MMPs activity and analysis of ECs secretory profile).
Gene expression was analyzed by qPCR and protein levels by Western blotting. To explain the differential cytotoxicity of the inhibitors, a comet assay as well as analyzes of the interaction of the inhibitors with DNA were performed using fluorescence spectroscopy, and the results were verified by in silico modeling.
All inhibitors effectively decreased H3cit in ECs and decreased cells migration formation of pseudocapillary structures. Importantly, we report a decrease in the production/secretion of the key angiogenesis regulator – VEGFA. Additionally, activation of the PI3K/Akt pathway was decreased, which may explain the antiangiogenic effects. Our results indicate that PAD inhibitors significantly modulate the functions of ECs, significantly reducing their angiogenic potential.

Inhibition of NETosis by PAD Inhibitors in a Whole Blood Assay: Reduction of Citrullinated Histone H3 Release

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Neutrophil extracellular traps (NETs) are web-like structures composed of decondensed chromatin and antimicrobial proteins that are expelled by neutrophils to trap and neutralize pathogens. NETosis, the process of NET formation, is triggered as a part of the immune response but can also contribute to inflammatory and autoimmune diseases when dysregulated.

Peptidylarginine deiminases (PADs) are enzymes that catalyze the conversion of arginine residues in proteins to citrulline, a process known as citrullination. This modification is critical for chromatin decondensation during NETosis. The inhibition of PADs has been shown to reduce NET formation by preventing the citrullination of histone H3.

In our study, we investigated the effect of PAD inhibitors on NETosis in a whole blood assay. Using an ELISA, we demonstrated that PAD inhibitors effectively reduced the release of citrullinated histone H3, a marker of NET formation. Our findings highlight the therapeutic potential of PAD inhibitors in controlling aberrant NETosis and its associated pathological conditions.

Non-cell-autonomous regulation of cell reprogramming through extracellular citrullinated chromatin

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The discovery that somatic cells can be reprogrammed to a pluripotent state and instructed to differentiate into various cell types promises to revolutionise regenerative medicine. However, the molecular mechanisms that underlie reprogramming and cell fate decisions are incompletely understood, hampering the development of rational approaches to promote cell reprogramming. Previous work has implicated the peptidylarginine deiminase 4 (PADI4) in the regulation of pluripotency during the reprogramming of somatic cells into induced pluripotent stem (iPS) cells. Using transcription factor-driven reprogramming of neural stem cells, we show that widespread histone citrullination within reprogramming cultures precedes the expression of pluripotency genes and the formation of iPS colonies. PADI4 is expressed exclusively in cells that do not reprogramme and citrullinated chromatin is found in the extracellular space surrounding the emerging iPS colonies. Pharmacological inhibition of PADI4 or extracellular chromatin sensing pathways reduces reprogramming efficiency, suggesting that PADI4 and citrullinated chromatin support reprogramming in a non-cell-autonomous manner. Extracellular citrullinated chromatin is also detected in mouse tissues upon the activation of transgenes expressing the reprogramming transcription factors as well as in regenerative blastema, suggesting that it may act as a cell communication mechanism mediating cell fate transitions also in vivo. Our work uncovers a novel mechanistic principle of cell reprogramming and cell fate control and has the potential to open new approaches for improving reprogramming efficiency and tissue regeneration.

Exploring Motif Preferences and Functional Specificity of the Protein Arginine Deiminase Family

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The conversion of arginine to citrulline in proteins is catalyzed by protein arginine deiminases (PADs). In humans, there are five PAD isozymes with different tissue and subcellular expressions, resulting in different access to substrates. Although it is known that PAD enzymes differ in their protein target spectrum, the influence of substrate sequence motif and its origin are not yet elucidated. To study the motif preferred by the different PAD enzymes, cell lysates were subjected to citrullination by PAD1-4 *in vitro*, digested with trypsin, fractionated and measured by LC-MS/MS to obtain a deep and comprehensive survey of the citrullination landscape. Using this setup, an average of over 90,000 peptides (~6100 proteins) could be quantified per sample. The extent of citrullination achieved by PAD1-4 ranged between 6-23%, with 8,000-22,000 individual citrullination sites confidently identified across triplicates. Notably, the number of sites generated by PAD1 and 2 was almost double than that of PAD3 and 4. Motif analysis of the citrullinated peptides revealed a distinct preference of both PAD3 and 4 towards D in -1 and D, E, G, and N in +1 position relative to the modified R. PAD1 and 2 showed a more permissive motif, favoring K, D, E and I in the entire region around the citrullination site. To further investigate this difference in substrate preference, we performed site-directed mutagenesis on PAD4, targeting five residues (Q346, R374, G403, R639 and H640) around the substrate binding sites that differ between PAD2 and PAD4. Most of the mutants showed elevated catalytic activity compared to the wild type and increasing similarity in their substrate motif to that of PAD2. Specifically, Q346, R639, and H640 residues were found to play important roles in the substrate selection of PAD4. This work presents the first systematic investigation of motif preferences of PAD1-4 enzymes in the human proteome and identifies key residues involved in substrate selection in PAD4

Exploring the functions of histone citrullination in modulating stem cell behaviour

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Citrullination of histone occurs on arginine residues. Catalyzed by the peptidyl arginine deiminase family (Padis), this modification alters the charge of the histone tail and modulates its interaction with DNA and chromatin interacting proteins. Although a growing number of studies demonstrated the gene regulatory role of histone citrullination in developmental processes and cancer progression, and revealed its cross-talk with various histone modifications, its characteristics at the genome-wide level are unclear. This study employs proliferating and differentiated mouse keratinocytes as models, aiming to examine the genome-wide enrichment, dynamics, and function of histone citrullination in the context of cell differentiation.

To start, ChIP-seq targeting to H3R2+8+17Cit, and H3R8Cit have been conducted. However, the enrichment of these citrullination marks is highly similar to total histone H3, and no differential enrichment was observed between cell states. These results suggest the existence of technical hurdles in profiling H3Cit or that these marks are persistent and unchanged during differentiation. Currently, cells depleted for multiple Padis are being generated by CRISPR to test for H3Cit ChIP antibodies specificity, and to examine whether Padis depletion impact keratinocyte differentiation.

Besides H3Cit ChIP-seq, Padi4 binding on the chromatin will be profiled to indirectly predict a subset of citrullinated loci. In parallel, ATAC-seq, RNA-seq, and ChIP-seq targeting the reported H3Cit-associated histone marks will be conducted for integrative analysis.

Moreover, our previous finding suggests that Padi4 regulates translation, restricting proliferation and lineage commitment of hair follicle progenitors. Diving deeper into the mechanisms, immunoprecipitation-mass spectrometry (IP-MS) will be performed to identify Padi4 interactors. Also, Padi4-associated chromatin and transcription factors will be revealed to understand its epigenetic regulations.

Down-regulation of human PAD1 in a three-dimensional reconstructed epidermis affects nucleophagy and epidermal barrier function.

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In normal human skin, three peptidylarginine deiminases (PAD1-3) are expressed. To analyze PAD1 involvement during keratinocyte differentiation, this isotype was down-regulated by an RNA interference approach in a three-dimensional reconstructed human epidermis (RHE) model. During normal RHE production, from day 4 to day 10, the deimination rate increased by a factor of ~ 14 and PAD1 relative mRNA level by a factor of ~8. Like in interfollicular epidermis, deiminated proteins were mainly immuno-detected at the stratum corneum level. Down-regulation of PAD1 in this model, using two distinct shRNAs induced a reduction of keratohyalin granule area, a clear reduction of filaggrin monomer amount, a decrease of the number of corneocyte layers, and a quasi- full extinction of the protein deimination without major impact on keratinocyte proliferation. PAD1 deficiency altered the outside/in permeability of the epidermal model and induced the collapse of the trans-epidermal-electric resistance values without impact on the superficial pH or the trans-epidermal water-loss. At the protein and transcript levels, detection of cornified envelope constituents and autophagic markers was not affected but several tight junction actors were modulated and transglutaminases 1, 3 and 5 falled down. Furthermore, PAD1 deficiency also altered the shape of nuclei in the granular layer of RHEs, with deep invaginations of the nuclear membrane and a huge increase of perinuclear vesicles, as shown using transmission electron microscopy. Treatment of normal RHEs, from day 8 to day 10, with Cl-amidine, a pan PAD inhibitor, confirmed the involvement of PADs in nucleophagy. Therefore, PAD1 deficiency affects the epidermal barrier organization and function. The present work is also the first demonstration that PAD1 and deimination play a major role in the nucleophagy process associated with the late steps of keratinocyte differentiation.

Deimination of filaggrin improves its proteolysis in the epidermis.

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In the normal human epidermis, filaggrin monomers are deiminated, mainly by PAD1, before their full degradation to free amino acids, a process also known as filaggrinolysis essential for the epidermal barrier functions. This proteolysis involves several peptidases, namely caspase-14, calpain-1 and bleomycin hydrolase. However, their combined activities do not explain the complete proteolysis of filaggrin, and other proteolytic enzymes should be implicated. Mining several omics analyses we identified the prolyl-endopeptidase PREP as a candidate peptidase to act during filaggrinolysis. In normal human skin, PREP co-localizes with filaggrin in the upper epidermis as demonstrated by indirect immuno-fluorescence confocal microscopy.
Tandem mass spectrometry and fluorescent quenching activity assays demonstrated that PREP cleaved several synthetic peptides derived from the filaggrin sequence, at the carboxyl side of internal proline residues. Deimination of an arginine close to the cleavage sites increased PREP enzymatic efficiency. In tridimensional reconstructed human epidermis, specific PREP inhibition using benzyl-oxy-carbonyl-prolin-prolinal (or ZPP) induced the accumulation of FLG monomers.
Since we, and others previously demonstrated that the deiminated form of filaggrin is more sensitive to the proteolytic activity of calpain-1 and bleomycin hydrolase, deimination appears as a major step to facilitate the full proteolysis of filaggrin in the epidermis. The decreased expression of PAD1 and deimination of filaggrin in the skin of atopic dermatitis patients may therefore participate to the pathogenesis of this frequent inflammatory skin disease.

Development of a Mass Spectrometry-Compatible Chemical Probe for Protein Citrullination Enrichment

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A systematic exploration of citrullination at the proteome-wide level advances the understanding of its role in health and disease. Although mass spectrometry (MS) is a reliable and sensitive approach to pinpoint the citrullination sites, the abundance of citrullination is low, requiring enrichment to identify these sites comprehensively. Enrichment probes utilizing the reaction of glyoxal and citrulline provide specific enrichment of citrullinated proteins and peptides. However, this often results in lower fragmentation efficiency or missing site-specific information in MS. Here, we present an MS-compatible and cleavable chemical probe for the enrichment of citrullinated peptides, based on two commercially available compounds, making it readily accessible to the community to explore functional implications in diverse biological contexts. Citrullinated peptides were derivatized by 4-azidophenyl glyoxal, followed by a click reaction with dde-biotin alkyne that adds a biotin residue with a cleavable linker. After enrichment by streptavidin beads, the peptides are cleaved off, leaving a mass tag of 212 Da. The peptides were then measured in LC-MS/MS and analyzed by MSFragger using a custom variable modification. First, synthetic citrullinated peptides were used to optimize reaction conditions and spiked into a tryptic Hela digest to assess platform sensitivity in a complex background. The detection limit of a single peptide is ~780 fmol. Next, using an in vitro citrullinated Hela sample, the number of identified citrullinated peptides more than doubled after enrichment, with intensity-based enrichment of up to 90% for high citrullination content. Finally, extending its high-throughput capabilities in a 96-well format demonstrated reproducible quantitation in technical replicates. In conclusion, our method enhances the depth of the citrullination proteome and enables high-throughput application of citrullination analysis in clinical samples.

Peptidylarginine deiminase 4 (PAD4) is a key factor for SARS-CoV-2 replication and SARS-CoV-2-induced pro-inflammatory responses

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Background: The novel coronavirus SARS-CoV-2, responsible for the COVID-19 outbreak, has become a pandemic, threatening millions of lives worldwide. Despite the plethora of studies that have advanced our understanding of the SARS-CoV-2 infection many mechanisms remain to be better understood in order to control the virus's spread and treat COVID-19 clinical cases. Peptidyl-arginine deiminases (PADs) are a family of cellular enzymes which dysregulation leads to an aberrant citrullination (a post translational modification) which is a biomarker of several inflammatory conditions. Based on some similarities between the clinical outcome observed in autoinflammatory disease and COVID-19, including lung involvement and abnormal cytokine release, this study aimed to evaluate the antiviral activity of PAD inhibitors in vitro and in vivo, as well as their ability to mitigate pro-inflammatory reactions. Methods: Using different SARS-CoV-2 strains, we tested the antiviral and anti-inflammatory activity of PAD inhibitors in human cell lines and in K18-hACE2 transgenic mice. We used qPCR to quantify viral genomes, Western blot analysis to evaluate the expression of viral proteins, plaque assay to evaluate the production of new virions, and ELISA to evaluate cytokines production. Furthermore, we assessed PAD4 expression and the pattern of protein citrullination upon infection.

Results: SARS-CoV-2 infections were significantly associated with PAD-mediated citrullination in vitro and with a specific increase in PAD4 expression. Moreover, the pharmacological inhibition of the PAD4 enzyme led to a significant reduction of viral replication and pro-inflammatory mediators.

Conclusions: Our results suggested that SARS-CoV-2 induces an increase in citrullination and that this increase plays a key role in COVID-19 pathology. All in all, the ability to modulate both of these features would make PAD-inhibitors promising candidates for the control of the COVID-19 disease.

Understanding histone citrullination as an epigenetic modulator.

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Eukaryotic DNA is complexed with histone proteins to form nucleosomes. In turn, nucleosomes form chromatin, a highly organised macromolecular structure that allows DNA to exist within the confines of the nucleus. The execution of DNA-based processes, such as replication or transcription, necessitates regulation of its accessibility within chromatin. Therefore, chromatin structure is highly dynamic. A central mechanism of chromatin regulation is through histone post-translational modifications (PTMs). The presence of PTMs is governed by proteins that deposit or remove them (epigenetic writers or erasers), while their downstream functions are carried out by proteins that bind them (epigenetic readers) and mediate a downstream response. Histone PTMs function either individually, or in combination with adjacent marks (histone code hypothesis).

Histone citrullination is a well-established modulator of transcription and chromatin accessibility, however our understanding of the molecular mechanisms through which it operates is in its infancy. Specifically, it is unknown whether epigenetic readers exist that are specific to citrulline marks. Previous studies have demonstrated that the presence of citrullines affects the recruitment of readers to nearby trimethyl-lysines, suggesting that citrulline marks operate within the histone code.

The N-terminal tail of histone H3 is subject to a plethora of PTMs and includes a number of citrullination sites, as well as neighbouring arginine-lysine residues. We will use protein biochemistry and Mass Spectrometry to identify epigenetic readers to histone H3 citrullines and understand the cross-talk between citrullines and adjacent lysine trimethyl and acetyl marks. Our ultimate aim is to understand how citrulline readers and arginine-lysine modification crosstalk function in the context of cancer and stem cell biology.

PAD3 autocitrullination and the structure

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Peptidylarginine deiminases (PADs) are enzymes that catalyze the Ca2+-dependent conversion of arginine residues into proteins to citrulline residues. Recently, we determined the structures of PAD3 in six states. Among these, we identified a “nonproductive” form of PAD3 in which the active site was disordered even though five Ca2+ ions were bound. This strange structure was probably obtained as a result of either high Ca2+ concentration (~260 mM)-induced denaturation during the crystallization process or high Ca2+-concentration-induced autocitrullination. While autocitrullination has been reported in PAD2 and PAD4 for some time, only a single report on PAD3 has been published before our work. In this meeting, I will report that PAD3 catalyzes the autocitrullination reaction. I will also report that the autocitrullination sites increase depending on the Ca2+ concentration and reaction time. These findings suggest that some of the arginine residues in the “nonproductive” form of PAD3 would be autocitrullinated. Furthermore, most of the autocitrullinated sites in PAD3 were located near the substrate-binding site. Given the high Ca2+ concentration in the crystallization condition, it is likely that Arg372 was citrullinated in the “nonproductive” PAD3 structure, the structure was slightly altered from the active form by the citrulline residue, and probably inhibited Ca2+-ion binding at the proper position. Following Arg372 citrullination, PAD3 enters an inactive form; however, the Arg372-citrullinated PAD3 are considered minor components in autocitrullinated PAD3 (CitPAD3), and CitPAD3 does not significantly decrease the enzyme activity. Autocitrullination of PAD3 could not be confirmed at the low Ca2+ concentrations seen *in vivo*.

Elucidating the role of PADI4 in p53-mediated tumor suppression

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TP53 is a tumor suppressor gene that is the most frequently mutated gene in human cancer. It's protein product, p53, is a transcription factor that is activated in response to genotoxic and cytotoxic stress to transcriptionally coordinate over 3000 genes involved in cell cycle arrest, DNA repair, apoptosis, metabolism, and autophagy. Since p53 has so many diverse roles in protecting the integrity of the genome and maintaining homeostasis, several mechanisms exist to regulate and fine-tune p53 functions. Post-translational modifications influence p53 activity by affecting its stability, localization, and protein-protein interactions. Using mass spectrometry, I have identified citrullination as a novel post-translational modification on p53, and peptidyl arginine de-iminase type 4 (PADI4) has been identified as the enzyme responsible for the nonreversible conversion of arginine to citrulline on p53. We found that PADI4 is a p53 target gene, that it fails to be transactivated by tumor-associated p53 hypomorphic variants studied in our lab, and that overexpression of PADI4 results in a tumor suppressive phenotype both in vitro and in vivo. We performed ChIP-seq on PADI4-induced and p53-stabilized cells and found that PADI4 and p53 colocalize at the promoter region of a small subset of p53 target genes that regulate metabolism and autophagy, including PRKAB1(AMPK), and PTEN. A regulatory network analysis of our data confirmed that a large majority of these target genes are also co-regulated by the stress-induced activating transcription factor 3 (ATF3). Using a genome browser, I have identified ATF3 binding motifs near the promotors of PRKAB1, and PTEN. My data support the hypothesis that PADI4 binds to p53 and citrullinates it, resulting in enhanced binding to promoter regions near ATF3 binding sites, potentially through altered protein-protein interactions with ATF3. This work has the potential to uncover new pathways that regulate tumor suppression by p53.

A novel mass-spectrometry-based approach enabling site-specific detection of plasma protein citrullination in inflammation-induced thrombosis

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Citrullination of arginine residues by peptidyl arginine deiminase (PAD) enzymes drives autoimmune diseases. This post-translational modification was recently shown to inactivate ADAMTS13 and SERPINs which suggests involvement of citrullination in inflammation-related thrombosis. However, citrullination is notoriously difficult to detect by conventional methods, especially in complex proteomes. Here, we developed a novel citrulline probe which enables enrichment of citrullinated peptides prior to site-specific analysis via mass spectrometry (MS). We utilized this method to analyze citrullination of fibrinogen by PAD4 and show the utility of this probe for assessment of plasma protein citrullination. The citrulline probe was synthesized and its specificity was assessed by NMR. Highly purified PAD4 was used for in vitro citrullination of fibrinogen. Following tryptic digestion, citrullinated peptides were labelled with the probe. Subsequently, citrullinated peptides were enriched and analyzed by MS. Probe-specific peptide mass shift was used for reliable identification of citrullination sites using MaxQuant. The newly developed probe selectively modifies citrulline. Using a time-course of PAD4-incubated fibrinogen we measured an increasing intensity and number of citrullinated fibrinogen residues with a maximum of 52 sites. Clustered fibrinogen alpha-chain residues Arg218, Arg271, Arg458, Arg573 and Arg591 are preferentially citrullinated. Applicability of our method for complex proteomes was showcased by measuring a dose-response curve of spiked citrullinated fibrinogen in human plasma and plasma samples derived of patients with severe COVID-19. Our findings indicate that PAD4 preferentially modifies specific sites within fibrinogen that overlap with known targets of anti-citrullinated protein antibodies (ACPA). In addition, our findings provide evidence for extensive protein citrullination in patients with COVID-19.

Structural insight into the function of human peptidyl arginine deiminase 6

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Peptidyl arginine deiminase 6 (PADI6) is vital for early embryonic development in mice and humans, yet its function remains elusive. PADI6 is less conserved with the other PADIs, and it is currently unknown whether it has a catalytic function. Towards understanding the function of PADI6, we have determined a 2.44 Å crystal structure of human PADI6, the first high resolution structure of the wild type protein.

We show that human PADI6 dimerises like hPADIs 2-4, however does not bind calcium ions and is inactive in in vitro assays against standard PADI substrates. Our crystal structure reveals that hPADI6 is structured in the absence of calcium ions where hPADI2 and hPADI4 are not, and that the Ca-binding sites are not conserved. Moreover, our structure reveals that whilst the key catalytic aspartic acid and histidine residues are structurally conserved, the cysteine is displaced far from the active site centre. The hPADI6 active site pocket appears closed through a unique evolved mechanism in hPADI6, not present in the other PADIs. Finally, with a high-resolution X-ray crystal structure of hPADI6 in hand, we investigated the structural consequences of the reported clinically significant variants by modelling biallelic structural damage scores for variants reported to cause infertility, hydatidiform mole formation, and multi-locus imprinting disorders. We show that variants that result in infertility have a higher structural damage score than those that result in imprinting disorders and highlight three single amino acid substitutions reported to cause infertility that indicates PADI6 dimerisation is critical for its function.

Taken together, these findings provide insight into how the function of hPADI6 may differ from the other PADIs based on its structure and provides a resource for characterising the damaging effect of clinically significant PADI6 variants.

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progress happens together
abcam

Abcam is a global life science company supporting customers at the forefront of life science research. At Abcam, we believe the scientific community goes further, faster when we go there together.

And to keep on making ground-breaking discoveries, we need to work together in new ways. That's why we're constantly innovating to help scientists drive their research forward by providing products and solutions that play an essential role in fundamental research, drug discovery, diagnostic and therapeutic applications.

We started with a simple mission: to provide the best biological reagents to life scientists worldwide. Today, we help 750,000 researchers in over 130 countries deliver faster breakthroughs in areas like cancer, neurological disorders, infectious diseases, and metabolic disorders.

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Cayman helps make research possible by providing products and services to scientists worldwide. Our collection includes high-quality biochemicals, assay kits, antibodies, and proteins, with a dedicated focus on products related to citrullination in health and disease.

We provide a wide range of research tools to study citrullination and related processes. Citrullinated proteins such as histone H3, vimentin, α-enolase, and fibrinogen, their native counterparts, and monoclonal and polyclonal antibodies are available for these targets. We offer recombinant proteins, antibodies, ELISA kits, inhibitors, and inhibitor screening assays to study PAD enzymes. To support NETosis research, Cayman has several assays available, including the NETosis Assay Kit, the Citrullinated Histone H3 ELISA, and the NETosis Imaging Assay Kit. Custom services for protein and antibody production, NET formation and analysis, and structure-based drug design are also available.

Introduce yourself to Micah Doty, a Scientist in Cayman's Antibody R&D and Services Division, at the EMBO Workshop or visit www.caymanchem.com for more information

https://www.caymanchem.com/

The Starlab group specialises in the field of Liquid Handling Technology and laboratory products.

Our products and services are in daily use at research institutions in biological and medical institutes, universities and hospitals, as well as in companies in the life sciences. We have earned an excellent reputation within the research community as a consultative company who takes a structured approach to Liquid Handling products and support. We work very closely with our customer network to continuously improve the products we offer, and to seek out new opportunities both from a product and support perspective. We have become a significant market player in all the product and service areas where we focus our efforts.

Our success is due to our highly-trained and motivated staff and a portfolio of high quality products and services.
Starlab are liquid handling experts, with many years' experience in automated injection moulding processes for the manufacture and sale of high quality laboratory consumables for research and development. With TipOne® we have developed one of the world's leading pipette tip systems. Quality, price and service are Starlab's core principles

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QIMA Life Sciences is at the forefront of contract research and innovation in the cosmetics, dermatology, pharmaceutical, and biotech industries. Our mission is to support biotech firms, pharmaceutical laboratories, and leading cosmetic brands in their pursuit of new drug candidates and validated product claims.

Our division offers comprehensive testing solutions across all development phases of cosmetic and pharmaceutical products. This includes bio-prediction using artificial intelligence to forecast molecule effects, pre-clinical in vitro and ex vivo efficacy tests through sophisticated biological models, and clinical evaluations via advanced skin and hair imaging and bioanalysis of clinical samples. By leveraging innovative methodologies, we contribute significantly to our clients' endeavors for a healthier future.

Behind our success is a network of 5 labs—four located in France and one in Germany—staffed by 150 dedicated professionals, including 34 PhDs. Our team's expertise and commitment to innovation and client satisfaction ensure that our services are distinguished by scientific integrity and exceptional quality.

Recent expansions in our French laboratory have not only enhanced our technical capabilities but also improved work conditions and minimized environmental impact, reflecting our dedication to sustainable practices. Our commendable Silver EcoVadis ESG rating underscores our commitment to environmental and social responsibility.

As we look to the future, QIMA Life Sciences is focused on integrating synergistic companies, adopting cutting-edge technologies, and adhering to stringent environmental standards. Our growth strategy is aligned with the evolving needs of the biotechnology and pharmaceutical sectors, ensuring global support for our clients.

For more information about QIMA Life Sciences and our services, please visit our website (www.qima-lifesciences.com) or contact us directly at ls@qima.com.

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Our Partners ...

Fondation Toulouse Cancer Santé - Innabiosanté is a private foundation created by:

- Amgen
- GSK
- Pierre Fabre
- Siemens
- Total Energie
- with the participation of the French National Research Agency (ANR).

Fondation Toulouse Cancer Santé (Fondation TCS) has been state approved since 2006. Its specific aim is to promote public and private research partnerships by financing interdisciplinary research projects in Toulouse area.

Fully dedicated to research in basic sciences applied to health domain and oncology in particular, Fondation Toulouse Cancer Santé selected 60 projects in total, 7 technological platforms, three research chairs:

- Chair of Bio-informatics in Oncology at the CRCT – Fondation TCS / Inserm / Pierre Fabre Research Institute
- Oncobreast Chair – research chair on breast cancer funded by Total Energies SE / Fondation TCS / Inserm & Oncopole Claudius Regaud
- Cristal Unlimited Chair - research chair on Acute Myeloid Leukemia funded by Foundation Unlimited and Fondation TCS

This represent more than 16 millions euros involvement in Toulouse research laboratories.

https://www.toulousecancer.fr/

Mercure Saint-Georges hotel

The Mercure Toulouse Centre Saint-Georges invites you live like a local. In the heart of the « Ville rose », restaurants, shops, museums and transport are just a short walk away.

The trendy hotel has meeting rooms, spacious family rooms and apartments. Why not sip on a cocktail at Le Mahogany Club bar during your stay. When the days get warmer, head to our shady terrace for drinks by the swim lane.

Mercure Toulouse Centre St Georges is right in the historic center near the Place du Capitole, Augustinians Museum, Jacobins and Basilica of St Sernin, a must-see stop on the Santiago de Compostela Way. Ideal for visiting the region's sites: Albi and its cathedral, Carcassonne castle, the medieval villages of Cordes-sur-Ciel and Moissac, the Dordogne valley and its gastronomy, Lourdes and Rocamadour.

At Mercure Toulouse Centre St Georges, discover and enjoy everything close by. Science lovers? visit the City of Space, Aeroscopia and Airbus; Love nature? stroll along the Canal du Midi or the Garonne.

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Welcome to

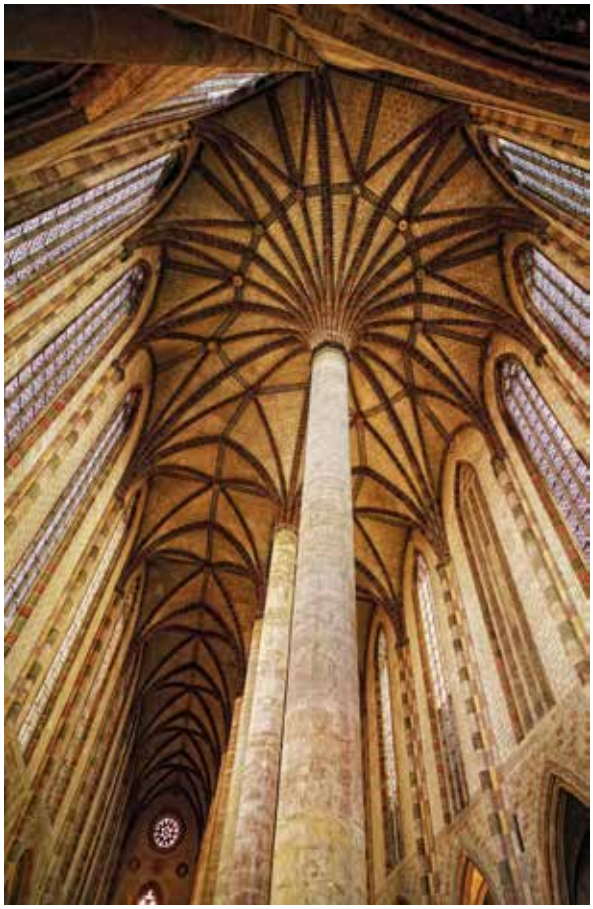
...the “Ville Rose”

Warm, spontaneous, passionate, Toulouse is the capital of Occitania. Once the capital of the Visigoths and now the capital of the air and space industry, Toulouse invites you to explore its 2000 years of history. Its churches, cathedral and basilica recount the Roman origins and religious influence of Toulouse through the ages.
The mansion houses call to mind the Golden Age of the Renaissance and the pastel trade. Toulouse, an aviation pioneer, is today Europe’s leading centre for aeronautics and home to some exceptional sites that absolutely have to be visited, such as Airbus with its visit of the A380 and Concorde or even the Cité de l’Espace.

Three major landmarks are listed as UNESCO World Heritage Sites: the Canal du Midi, the Basilica of Saint-Sernin and the Hôtel Dieu, a major stopover on the Way of St James.

Situated at the heart of everyday life in Toulouse and on the banks of the River Garonne, the Hôtel-Dieu Saint-Jacques is emblematic of a history of hospitality that is incredibly rich and enduring. It houses the headquarters of the Hospitals of Toulouse, the head office of the University Hospital Centre of Toulouse and the centre for skin research of the Pierre Fabre dermocosmetics laboratory.

After seeing the Place du Capitole, the basilica of Saint-Sernin and the Jacobins church, from the banks of the River Garonne you can admire the splendid views of the most iconic monuments of Toulouse.



ipbs TOULOUSE CANCER INFECTION INFLAMMATION

EMBO

EMBO Workshop

Mechanisms of Citrullination Regulation in Health and Disease