AHR'24

6th International AHR Meeting: Research, Prevention, Therapy

12 - 15 June 2024

Clayton Hotel Düsseldorf, Germany

Conference Program & Abstracts

Organized by IUF | LEIBNIZ RESEARCH INSTITUTE FOR ENVIRONMENTAL MEDICINE

6th International AHR Meeting: Research, Prevention, Therapy

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SCIENTIFIC COMMITTEE

Xavier Coumoul *(Paris, France)*
Charlotte Esser *(Düsseldorf, Germany)*
Thomas Haarmann-Stemmann *(Düsseldorf, Germany)*
Jean Krutmann *(Düsseldorf, Germany)*
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Jean Krutmann
Doreen Reichert
Katharina Rolfes

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INFORMATION

Abstracts

- Abstracts of speakers' presentations (ordered by program ID numbers O1 - O49)
- Abstracts of posters on display (ordered by ID-numbers P1 - P72)

Speakers

- **Speakers are kindly asked to upload their Power Point presentations at least 30 minutes before the respective session starts using the computer next to the conference registration desk**
- Invited speakers have 20 minutes time for presentation and 5 minutes for Q & A.
- Short oral speakers have 10 minutes time for presentations and 3 minutes for Q & A.
- 7-minute talks are followed by 1 urgent question. The respective posters will be displayed in the Da Vinci room with corresponding numbers (O39 – O45).
- Due to a tight schedule and to allow sufficient time for discussions, please adhere to the timing requirements.
- The best short oral presentation will be awarded with the ***Biochemical Pharmacology*** **Prize for the best short oral communication** (500 €).

Poster contributors

- Ensure your poster is displayed at the appropriate location and respect your poster number.
- Poster contributors are invited to stand by their poster during the poster session

    - <u>Odd numbered posters:</u>

      Thursday, June 13th 1:20 PM - 2:10 PM
      Friday, June 15th 3:50 PM- 4:40 PM

    - <u>Even numbers:</u>

      Thursday, June 13th 3:50 PM – 4:40 PM
      Friday, June 15th 1:20 PM– 2:10 PM

- Please dismantle your poster on Friday, June 15th by 6:00 PM.
- The best poster contribution will be awarded with the ***Biochemical Pharmacology*** **Prize for the best poster presentation** (500 €).

Meeting report

A report on the many exciting new developments in the field that will be presented and discussed at the 6th International AHR Meeting will be prepared by members of the Scientific Committee and published in the journal *Biochemical Pharmacology* (Elsevier).

WiFi

Free access to WiFi at “Clayton Hotel DUS”

Photobooth

Capture fun memories at our photobooth! Step in, strike a pose, and snap away with friends and colleagues. Our photobooth provides a delightful opportunity to create lasting memories of your time at the 6th international AHR meeting in Düsseldorf. Keep an eye out for the photobooth location!!!

Available time: 13th of June from 11:00 AM – 5:00 PM

Social Evening

Our social gathering will bring together individuals interested in the evolution of our species and transport them to the remarkable and picturesque **Neanderthal Museum**, located near the valley where Homo neanderthalensis remains were discovered.

- Guided tour of the museum, exclusively for our group
- Barbecue and beverages to follow
- Departure from the Clayton Hotel at approx. 6:15 PM
- Return: Departure from Neanderthal Museum at approx. 10:00 PM

What to Do in Düsseldorf on Your Free Evening

- Rhine Promenade: Enjoy a leisurely stroll along the Rhine River promenade and admire the skyline of Düsseldorf. Many cafes and bars offer stunning views of the river.
- Old Town ("The Longest Bar in the World"): Experience the vibrant nightlife in Düsseldorf's historic Old Town. Here you'll find a variety of cozy pubs, breweries, and clubs.
- Castle Tower and Burgplatz: Enjoy an evening stroll around Burgplatz and the Castle Tower. The illuminated castle complex provides a picturesque backdrop for photos and is a popular meeting spot for locals and visitors alike.
- Königsallee: Take a stroll along the famous Königsallee, an exclusive shopping street with luxury shops and boutiques. Even in the evening, the "Kö" offers a lively atmosphere and plenty of dining and drinking options.
- Rhine River Cruise: Take an evening boat cruise on the Rhine River and experience Düsseldorf from a different perspective. As you leisurely cruise along the river, you can admire the illuminated skyline and unwind for the evening.

Japan Quarter in Düsseldorf (location of the Clayton Hotel)

- Location: The Japan Quarter in Düsseldorf, also known as "Little Tokyo," is located in the city center, primarily centered around Immermannstraße and its surroundings.
- History: The quarter emerged in the 1950s as many Japanese companies began to establish themselves in Düsseldorf. Today, it stands as the largest Japan Quarter in Europe.
- Shopping: Immermannstraße is renowned for its variety of Japanese and Asians shops, including grocery stores, bookshops, fashion boutiques, and souvenir shops.
- Restaurants: Düsseldorf's Japan Quarter is famous for its authentic Japanese restaurants offering a wide range of dishes such as sushi, ramen, teishoku, and yakitori.
- Tourism: The Japan Quarter is a popular destination for tourists and locals alike seeking to experience Japanese culture and cuisine without traveling to Japan.

Please note that photographs and footage will be taken during the 6th International AHR Meeting 2024. If you do not want to be photographed, please inform the local organizer.

WELCOME

Dear Colleagues and Friends,

Welcome to the scientific conference on the aryl hydrocarbon receptor (AHR) here in the beautiful city of Düsseldorf! It is an extraordinary pleasure to welcome you all here, whether as old hands who have long been at home in the fascinating world of the AHR, or as new faces who recently joined the field.

It is both gratifying and amazing to see how this conference grew and developed since its inception in 2005. The diversity of participants and topics reflects the constant evolution and growing interest in AHR-related research. From established experts to emerging talent, from best practices to innovative approaches, we have gathered a rich mix of knowledge and experience waiting to be discovered and shared.

Düsseldorf welcomes you with open arms. This charming city on the Rhine not only offers an inspiring backdrop for our conference, but also a wealth of cultural and gastronomic experiences for you to sample. Take this opportunity to network successfully, exchange ideas and forge new collaborations.

We are particularly proud to welcome some of the leading minds in the field of AHR to share their insights and research findings and we expect exciting discussion. In addition, we have many talks selected from your submitted abstracts, and are thrilled that we can have a poster session of 79 poster presentations covering a wide range of research projects and results. We encourage you to explore the posters and engage in conversation with the authors. After all, it is often these informal conversations that lead to the most significant insights and inspiration.

We would like to express our heartfelt thanks to our friends of the scientific organizing committee, who supported us along the way and helped shaping the program. They will also help selecting the poster/short talk awards, which provide an important incentive for excellence in research.

Furthermore, we would like to express our sincere gratitude to the industrial companies, scientific societies, and the German Research Foundation (DFG), whose generous financial support has made this conference possible.

This is the third meeting taking place in Düsseldorf, and the 6th Meeting of its kind. Therefore, special thanks are also due to the IUF - Leibniz Research Institute for Environmental Medicine and its director Jean Krutmann for continued support and hospitality. We very much hope, that this meeting will be fun for all and further the understanding and therapeutic use of the AHR in the long term.

And if anything goes wrong – it is our fault. If all moves smoothly, it is the smiling universe!

Charlotte Esser & Thomas Haarmann-Stemmann

PAST CONFERENCES

- 2005, Sep 28-30 Düsseldorf, Germany (Charlotte Esser, Ellen Fritsche, Josef Abel, Jean Krutmann) **„Biochemistry and Function of the Arylhydrocarbon Receptor and other PAS-bHLH proteins"**

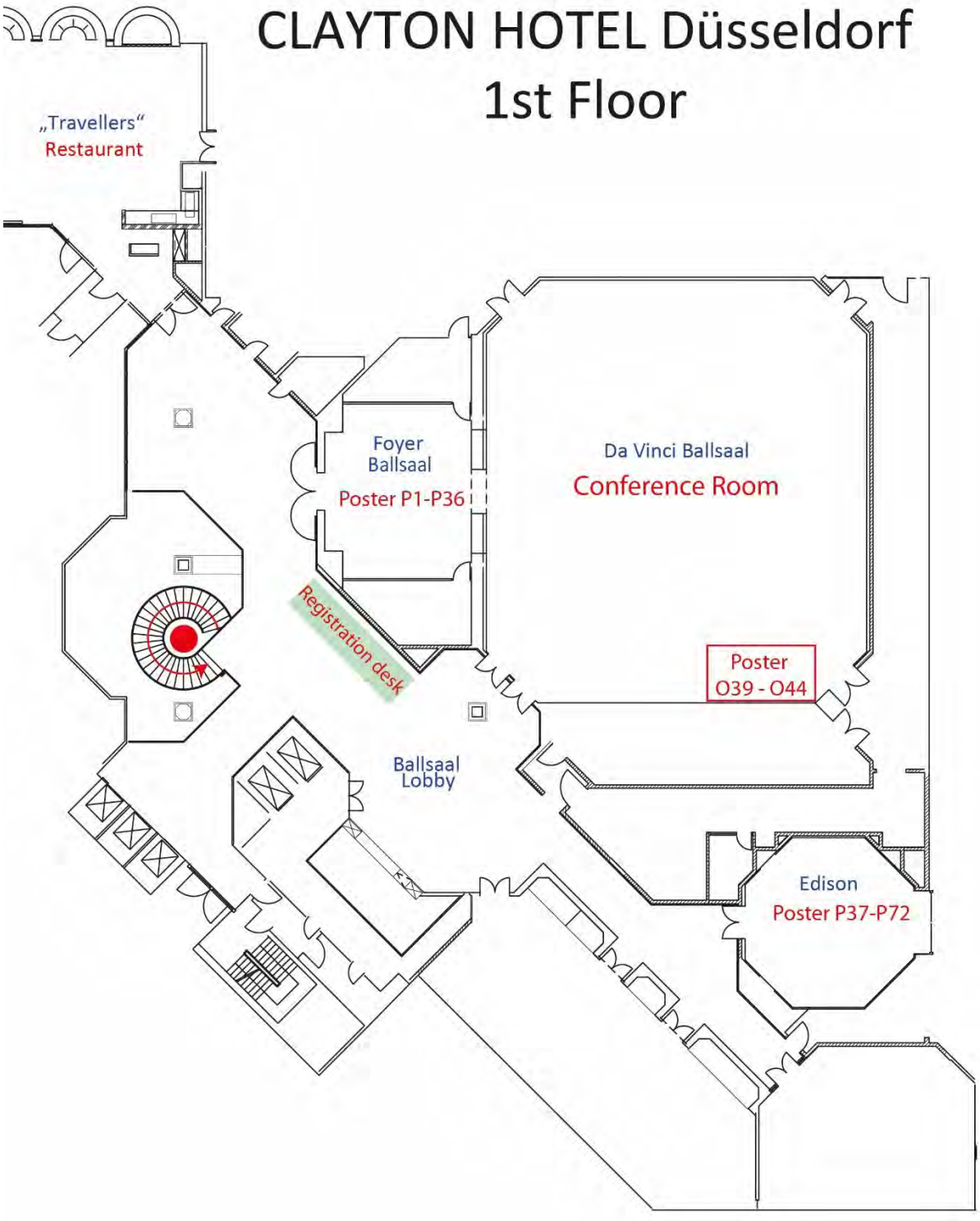
- 2011, Sep 21-24, Düsseldorf, Germany (Charlotte Esser, Jean Krutmann, Thomas Haarmann-Stemmann, Heike Weighardt, Irmgard Förster, Ellen Fritsche) "**Biology of the AHR"**
  Meeting report: DOI: 10.1007/s00204-012-0818-2

- 2016, Aug. 3-6, Rochester, NY, USA (B. Paige Lawrence, Alvaro Puga) "**The Aryl Hydrocarbon Receptor as a central mediator of health and disease"**

- 2018, Aug 28-31, Paris, France (Robert Barouki, Xavier Coumoul) "**AHR Meeting 2018**"
  Meeting report: DOI: 10.3390/Ijms19113603

- 2022, June 21-24, State College, PA, USA (Gary Perdew, Andrew Patterson) **"The Ah Receptor from Toxicity to Therapeutics"**
  Meeting report: DOI: 10.3390/ijms24065550

SITE MAP CLAYTON HOTEL



NOTES

PROGRAM

WEDNESDAY, June 12th

4:00 PM — **Registration desk open**

5:30 PM — **Welcome & Introduction**

Keynote lecture

Chairs: Charlotte Esser, Thomas Haarmann-Stemmann

The Ah Receptor as a model PAS sensor [O1]
Christopher Bradfield (Madison, WI, USA)

6:45 PM – 9.00 PM — **Welcome reception**

Drinks & finger food

THURSDAY, June 13th

8:00 AM — **Registration desk open**

9:00 AM — **Session 1: Molecular facets of AHR signaling**

Chairs: Paige Lawrence & Jörg Lehmann

Physiological AHR activity, a tryptophan metabolite receptor hiding in plain site [O2]
Gary Perdew (University Park, PA, USA)

Structural insights into the activation of human aryl hydrocarbon receptor by the environmental contaminant benzo[a]pyrene and structurally related compounds [O3]
William Bourguet (Montpellier, France)

Fine-tuning AhR activity: Lessons from studies on the ARNT isoforms[O4]
Casey Wright (Galveston, TX, USA)

Short talks selected from abstracts

Structural mechanism of the ligand-activated heterodimeric AHR-ARNT complex [O5]
Dalei Wu (Qingdao, China)

Targeting the Aryl hydrocarbon Receptor (AHR) : A deep learning approach for molecular activity prediction [O6]
Lianyi Han (Guangzhou, China)

10:40 AM — **Coffee break**

11:00 AM **Session 2: AHR in cancer & aging I**

Chairs: Christiane Opitz & Xavier Coumoul

The AhR is a Dominant Mediator of IFNγ Signaling Leading to Immune Checkpoint Up-regulation and Survival of Oral and Head and Neck Carcinomas [O7]
David Sherr (Boston, MA, USA)

Role of the AHR pathway in melanoma pathogenesis and treatment [O8]
Thomas Tüting (Magdeburg, Germany)

AhR as a regulator of innate and adaptive immunity in the tumor microenvironment [O9]
Tracy McGaha (Toronto, Canada)

Short talks selected from abstracts

Aryl hydrocarbon receptor promotes Alzheimer's disease development and progression through microglial modulation in the APP^{NL-F} knock-in mouse model [O10]
Carmen Nieto-Vaquero (Madrid, Spain)

Tonic activation of Aryl Hydrocarbon Receptor by food-derived ligands is essential for the efficacy of immune checkpoint blockade therapy [O11]
Elodie Segura (Paris, France)

12:40 PM **Lunch & poster session (1:20 PM; odd poster numbers)**

2:10 PM **Session 3: AHR in barrier organs**

Chairs: Sonja Fassbender & Caspar Ohnmacht

Exploration of the pathophysiological role of aryl hydrocarbon receptor in Langerhans cells at epicutaneous protein sensitization and psoriasis [O12]
Chih-Hung Lee (Kaohsiung, Taiwan)

Host and microbiota derived AhR agonist: role in intestinal homeostasis and IBD [O13]
Harry Sokol (Paris, France)

The anti-inflammatory topical medication tapinarof modulates mitochondrial metabolism and nucleic acid release [O14]
Rachel Clark (Boston, MA, USA)

Short talks selected from abstracts

Endothelial sensing of Aryl Hydrocarbon Receptor ligands regulates intestinal barrier immunity [O15]
Benjamin Wiggins (London, UK)

Aryl hydrocarbon receptor ligands from maternal microbiota shape skin barrier development [O16]
Mercedes Gomez de Agüero (Würzburg, Germany)

3:50 PM **Coffee break & poster session (even poster numbers)**

4:40 PM **Session 4: AHR ligands: Therapeutic approaches**

Chairs: Katharina Rolfes & Christoph Vogel

Targeting the aryl hydrocarbon receptor (AHR) with the selective inhibitor BAY 2416964 to revert cancer-associated immunosuppression [O17]
Gabriele Leder (Berlin, Germany)

Allosteric targeting of the AhR as a novel pharmacotherapeutic strategy [O18]
Zdenek Dvorak (Olomouc, Czech Republic)

Short talks selected from abstracts

Identification and in-vitro validation of new AHR ligands as potential therapeutics for inflammatory bowel diseases [O19]
Jörg Lehmann (Leipzig, Germany)

Antibiotic Modulation of the Gut Microbiome Ameliorates Hypertensive Organ Damage [O20]
Hendrik Bartolomaeus (Berlin, Germany)

6:00 PM **Free evening**

FRIDAY, June 14th

8:30 AM **Registration desk open**

9:00 AM **Session 5: AHR ligands: Adverse health effects**

Chairs: Mercedes Gomez de Agüero & Zdeněk Dvořák

The role of Aryl hydrocarbon Receptor and Polycyclic Aromatic Hydrocarbons in Combustion Particle Toxicity [O21]
Johan Ovrevik (Oslo, Norway)

A dynamic Aryl hydrocarbon Receptor (AhR) as a sensor of environmental pollutants [O22]
Christoph Vogel (Davis, CA, USA)

Short talks selected from abstracts

AhR and oxidative stress reporter mice as a tool for understanding transcriptional responses to Aroclor 1254 exposure across multiple generations. [O23]
G. Jean Campbell (Dundee, UK)

AhR-Mediated Impacts of BaP-Coated CeO_2 Nanoparticles on the Human Placental Barrier [O24]
Gaëlle Deval (Paris, France)

Exposure to airborne particulate matter increases the risk for atopic dermatitis: Clues for an involvement of AHR-dependent NADPH-dependent oxidoreductases [O25]
Frederick Hartung (Düsseldorf, Germany)

10:40 AM **Coffee break**

11:00 AM **Session 6: AHR in immunity I**

Chairs: Elodie Segura & Thomas Tüting

AHR and regulation of antiviral immunity [O26]
Paige Lawrence (Rochester, NY, USA)

Regulation and Function of the AhR in the context of type 2 immunity [O27]
Caspar Ohnmacht (Munich, Germany)

Role of AHR in the control of inflammation [O28]
Francisco Quintana (Boston, MA, USA)

Short talks selected from abstracts

AHR limits inflammation in intestinal macrophages [O29]
Nicola Diny (London, UK)

Breaking Aryl Hydrocarbon Receptor's Tumor Microenvironment Sensing for Enhanced cDC1-Mediated Antitumor Response [O30]
Marco Gargaro (Perugia, Italy)

12:40 PM **Lunch & poster session (1:20 PM; even poster numbers)**

2:10 PM **Session 7: AHR-related PAS proteins**

Chairs: Hatice Genç & Gary Perdew

Mice With HIF-1α/ AHR-Double Deficient Keratinocytes Share Similarities With UVB-Exposed Wildtype Littermates [O31]
Sonja Faßbender (Düsseldorf, Germany)

Role of the AhRR in inflammation and metabolism [O32]
Heike Weighardt (Bonn, Germany)

Short talks selected from abstracts

Exploring ligand-dependent interaction dynamics of bHLH-PAS receptors in real time: human AHR and the insect juvenile hormone receptor [O33]
Sarka Tumova (Ceske Budejovice, Czech Republic)

AHR Signaling and Cysteine Metabolism in Chronic Intermittent Hypoxia-Induced Hypertension [O34]
António Pimpão (Lisbon, Portugal)

3:30 PM **Coffee break & poster session (odd poster numbers)**

4:20 PM – 5:10 PM **Plenary talk**

Human-specific genetics: new tools to explore the molecular and cellular basis of human evolution [O35]
J. Gray Camp (Basel, Switzerland)

~ 6:00 PM **Social evening @ Neanderthal Museum**

SATURDAY, June 15th

8:30 AM **Registration desk open**

9:00 AM **Session 8: AHR in immunity II**

Chairs: Heike Weighardt & Francisco Quintana

AHR sensing microbial infections: a ticket to multiple destinations [O36]
Pedro Moura-Alves (Porto, Portugal)

Environmental influences via AHR on gut barrier regeneration and intestinal infection control [O37]
Brigitta Stockinger (London, UK)

Short talk selected from abstracts

AhR-microbiome interactions regulate intestinal IL-10R+ macrophages in non-obese diabetic mice [O38]
Allison Ehrlich (Davis, CA, USA)

10:05 AM **Session 9: AHR research: Emerging frontiers (Mixed pickles)**

Chairs: Charlotte Esser & Thomas Haarmann-Stemmann

7 x 7 min talks selected from abstracts

The Aryl Hydrocarbon Receptor as novel key driver of lipid metabolism and atherosclerosis [O39]
Rosana Huchzermeier (Aachen, Germany)

AhR Plasma Agonist Activity following Plant-Based versus Western Omnivorous Diets in Healthy Individuals [O40]
Laura de Boni (Cologne, Germany)

Identification and characterisation of the importin complexes involved in the nuclear translocation of the aryl hydrocarbon receptor [O41]
Jakub Gruszczyk (Montpellier, France)

Identification of AHR activity-modulating drugs with potential involvement in cutaneous adverse drug reactions [O42]
Alina Kuklinski (Düsseldorf, Germany)

Jun dimerization protein 2 (Jdp2) is a spatiotemporal transcriptional activator of the AhR via the Nrf2 gene battery [O43]
Kazunari Yokoyama (Kaohsiung, Taiwan)

Straightforward Access to Novel Aryl Hydrocarbon Receptor Modulators through a Designed Multicomponent Reaction [O44]
Ouldouz Ghashghaei (Barcelona, Spain)

Endogenous aryl hydrocarbon receptor ligands regulate blood-brain barrier integrity [O45]
Timo Mikel Rhiem (Düsseldorf, Germany)

11:05 AM **Coffee break**

11:25 AM **Session 10: AHR in cancer & aging II**

Chairs: Doreen Reichert & David Sherr

Exploring the crosstalk between tryptophan metabolism and the aryl hydrocarbon receptor (AHR) in estrogen receptor positive breast cancer [O46]
Christiane Opitz (Heidelberg, Germany)

Role of the AhR in Breast Cancer progression: a (micro)environment story… [O47]
Xavier Coumoul (Paris, France)

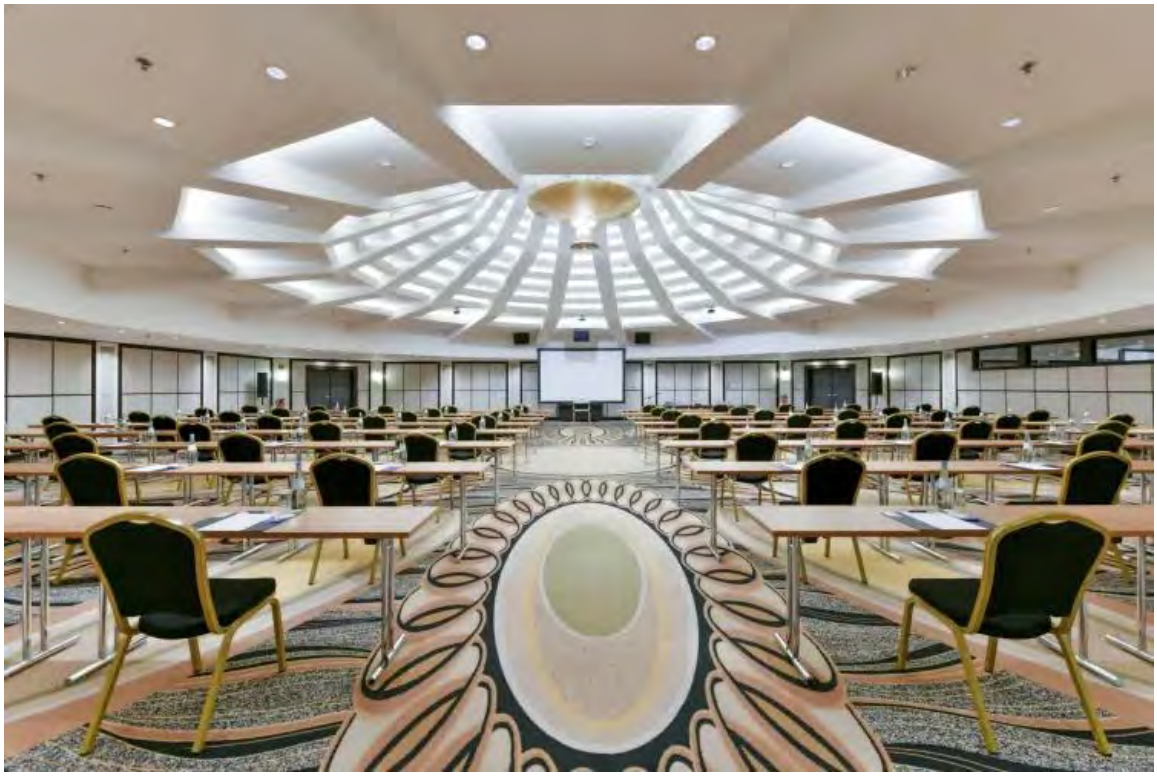
Short talks selected from abstracts

Investigating the role of the aryl hydrocarbon receptor on retinal function and morphology during aging and in age-related macular degeneration [O48]
Goldis Malek (Durham, NC, USA)

The tryptophan stress-induced mTORC1-AHR axis sustains autophagy and translation in tumors [O49]
Pauline Holfelder (Heidelberg, Germany)

12:45 PM **Award ceremony & farewell**

ABSTRACTS ORAL TALKS



INDEX OF SPEAKERS

[O1] The Ah Receptor as a model PAS sensor

Christopher Bradfield

The McArdle Laboratory for Cancer Research, Department of Oncology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, 53706, United States.

[O2] Physiological AHR activity, a tryptophan metabolite receptor hiding in plain site

Gary H. Perdew, Ethan W. Morgan, Iain A. Murray, Denise Coslo, and Andrew D. Patterson

Departments of Veterinary and Biomedical Sciences and Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, USA

The Ah receptor (AHR) plays a pivotal role in maintaining tissue homeostasis through regulation of cell differentiation, cell fate, barrier integrity and immune responses. However, considering these diverse activities, the regulation of basal AHR activity is poorly understood. AHR ligands can be found in the diet, as environmental contaminants, produced by the microbiota, and through host metabolism. Numerous tryptophan metabolites, including indirubin, indole-3-acetic acid, kynurenine and kynurenic acid, can activate the AHR in a species dependent manner. Considering the importance of AHR activity in homeostasis, it was hypothesized that systemic AHR activity would be best maintained by enzymatic production of ligands. Studies in germ-free mice revealed that a number of key tryptophan metabolites present in serum can activate the AHR and are not significantly influenced by the microbiota. This observation led to an overall assessment of the possible sources of tryptophan metabolism by the host. Tryptophan can undergo metabolism by tyrosine aminotransferase, glutamic-oxaloacetic transaminase, and interleukin 4-induced gene 1 to indole-3-pyruvate, an unstable intermediate. Subsequent metabolism of indole-3-pyruvate can lead to the production of AHR ligands, such as indole-3-acetic acid, indole-3-aldehyde, and indole-3-lactic acid. Importantly, these metabolites are not subject to a CYP1A1/1B1 mediated negative feedback loop, consistent with maintenance of homeostatic serum levels. These results have important implications in considering how the level of homeostatic AHR activity is driven during chronic conditions, such as cancer, diabetes, and stress, and how human genetic diversity may play a role in the level of circulating AHR ligands.

[O3] Structural insights into the activation of human aryl hydrocarbon receptor by the environmental contaminant benzo[a]pyrene and structurally related compounds

Hok-Sau Kwong[1], Matteo Paloni[1], Loïc Grandvuillemin[1], Savannah Sirounian[1], Aurélie Ancelin[1], Josephine Lai-Kee-Him[1], Marina Grimaldi[2], Coralie Carivenc[1], Claudia Lancey[3], Timothy J Ragan[3], Emma L Hesketh[3], Patrick Balaguer[2], Alessandro Barducci[1], Jakub Gruszczyk[1] and William Bourguet[1]

[1]CBS (Centre de Biologie Structurale), Univ Montpellier, CNRS, Inserm, Montpellier, France
[2]IRCM (Institut de Recherche en Cancérologie de Montpellier), Univ Montpellier, Inserm, ICM, Montpellier, France
[3]Leicester Institute of Structural & Chemical Biology and Department of Molecular & Cell Biology, University of Leicester, Lancaster Rd, Leicester, LE1 7HB, UK

The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor belonging to the bHLH/PAS protein family and responding to hundreds of natural and chemical substances. It is primarily involved in the defense against chemical insults and bacterial infections or in the adaptive immune response, but also in the development of pathological conditions ranging from inflammatory to neoplastic disorders. Despite its prominent roles in many (patho)physiological processes, the lack of high-resolution structural data has precluded for thirty years an in-depth understanding of the structural mechanisms underlying ligand-binding specificity, promiscuity and activation of AHR. We recently reported a cryogenic electron microscopy (cryo-EM) structure of human AHR bound to the natural ligand indirubin, the chaperone Hsp90 and the co-chaperone XAP2 that provided the first experimental visualization of its ligand-binding PAS-B domain. Here, we report a 2.75 Å resolution structure of the AHR complex bound to the environmental pollutant benzo[a]pyrene (B[a]P). The structure substantiates the existence of a bipartite PAS-B ligand-binding pocket with a geometrically constrained primary binding site controlling ligand binding specificity and affinity, and a secondary binding site contributing to the binding promiscuity of AHR. We also report a docking study of B[a]P congeners that validates the B[a]P-bound PAS-B structure as a suitable model for accurate computational ligand binding assessment. Finally, comparison of our agonist-bound complex with the recently reported structures of mouse and fruit fly AHR PAS-B in different activation states suggests a ligand-induced loop conformational change potentially involved in the regulation of AHR function.

[O4] Fine-tuning AhR activity: Lessons from studies on the ARNT isoforms.

Amy M. Cooper[1,2], Israel Muro[1], Madison G. Tanner[1,2], Luke A. Bourner[1,2], Barun K. Choudhury[1], Steven Widen[3,5], Kamil Khanipov[1], and Casey W. Wright[1,2,4,5]

[1] Department of Pharmacology and Toxicology; [2] Toxicology Training Program; [3] Department of Biochemistry and Molecular Biology; [4] Sealy Center for Environmental Health & Medicine; and [5] Gulf Coast Center for Precision Environmental Health, The University of Texas Medical Branch at Galveston, Galveston, TX 77555

Mounting evidence from my laboratory suggests that ARNT plays a pivotal role in shaping the output of an AhR response. While *ARNT* is constitutively expressed in most cell types, alternative splicing (AS) of *ARNT* pre-mRNA in immune cells produces ARNT isoform 1 and ARNT isoform 3, by the inclusion or exclusion of exon 5, respectively. In fact, naive T cells exhibit equal levels of ARNT isoform 1 and 3 whereas in T cell malignancies ARNT isoform 1 levels predominate. In turn, high ARNT isoform 1 levels dampen basal AhR signaling but augment NF-κB signaling leading to a growth and survival advantage. Conversely, ligand-induced AhR activation promotes unique CK2-mediated phosphorylation of ARNT isoform 1, relieving the inhibitory effects on AhR signaling but abrogating NF-κB activity. While our previous studies suggest that deregulated *ARNT* AS contributes to disease, whether *ARNT* AS has any bearing on normal cellular physiology and homeostasis is unknown. To investigate, we employed a T cell activation model given the vast number of genes that are alternatively spliced during this initial stage of T cell maturation. Indeed, we demonstrate that T cell activation stimulates *ARNT* AS whereby ARNT isoform 1 becomes the predominate isoform by twenty-four hours. Furthermore, using RNAi studies coupled with activation or inhibition of AhR signaling, we found that the rate of T cell activation is directly dependent on the temporally dominant ARNT isoform, which fine tunes AhR and NF-κB signaling to maintain a homeostatic T cell response. These observations support the targeting of specific ARNT isoforms for modulating T cell activity, and possibly T cell differentiation. Additionally, our results raise a tantalizing possibility that β-class PAS proteins wield significantly more control over α-class PAS proteins in an isoform-specific manner.

[O5] Structural mechanism of the ligand-activated heterodimeric AHR-ARNT complex

Xiaotong Diao[1], Dalei Wu[1]

[1] Shandong University, Qingdao, China

Aryl hydrocarbon receptor (AHR) is a vital transcription factor sensing and activated by a variety of exogenous and endogenous small-molecule ligands, which usually carry certain environmental or physiological signals. Upon ligand binding, AHR can translocate from cytosol into nucleus, dimerize with its obligate partner aryl hydrocarbon receptor nuclear translocator (ARNT), and initiate the downstream transcriptional programs. However, the molecular mechanisms of how AHR forms a heterodimer with ARNT and how AHR is promiscuously activated by different ligands have not been fully illustrated. Here we show the crystal structures of AHR-ARNT heterodimers bound by a panel of various ligands. These AHR-ARNT complex structures revealed a unique dimerization mode that differs from other fellow members of the basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) family. The ligand-bound PAS-B domain of AHR interacts with ARNT PAS-B domain, and together they form a relatively isolated architecture apart from their intertwined bHLH and PAS-A segments within the heterodimer. This structural independence of PAS-B domain correlates well with its known “inhibitory” role in AHR activation responsive to ligands. These results provide key information for our understanding of the regulatory mechanism of AHR transcriptional activity and future development of AHR-targeting drugs.

[O6] Targeting the Aryl hydrocarbon Receptor (AHR) : A deep learning approach for molecular activity prediction

Ziyan Sun[1,3], Lei Feng[1], Mingyuan Qin[1], Wei Li [2], Jiucun Wang[3], Jingjing Xia[1], Lianyi Han[1]

[1] Greater Bay Area Institute of Precision Medicine (Guangzhou), School of Life Sciences, Fudan University, Guangzhou, China
[2] Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, China
[3] State Key Laboratory of Genetic Engineering, Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Fudan University, Shanghai, China

The aryl hydrocarbon receptor (AHR) is crucial for modulating immune reactions, cellular growth, and the progression of tumors. Its potential for therapeutic applications has been firmly established through rigorous scientific studies, highlighting its appeal for pharmaceutical research and development. As technology has progressed and a variety of comprehensive datasets containing a multitude of cell types and ligands have become available, computational techniques, particularly those involving Artificial Intelligence, have emerged as essential instruments for the discovery of promising pharmaceutical agents. On the other hand, effectively modeling large-scale high-throughput screening (HTS) assays with accurate ligand numerical descriptors remains a challenging task, little has been made in utilizing AI for the identification of active compounds that interact with AHR. In our research, we have engineered a Graph Convolutional Network (GCN) tailored to forecast the activation potential of prospective small molecules that may interact with AHR. Our model utilizes data from the "Luminence-Based primary cell-based high throughput screening assay to identify activators of the Aryl Hydrocarbon Receptor (AHR)" conducted at The Scripps Research Institute Molecular Screening Center with 320 thousand small molecules screened. The preliminary result shows that the GCN model achieved 95% AUC, indicating that the model had a good ability to distinguish between positive and negative samples. The F1 score, precision and recall rate were all close to 90%. Furthermore, by applying molecular docking technology, we were able to predict the binding affinity of more than 7000 active small molecules with AHR proteins in our dataset. Cross-validation with GCN showed that 99% of these molecules demonstrated a strong affinity for AHR proteins, underscoring their significant potential as powerful activators.

In summary, the proposed Graph Convolutional Network (GCN) model for predicting the activation potency of small molecule candidates aimed at AHR has yielded encouraging outcomes in pinpointing therapeutic hits. The cross-validation of the GCN model with molecular docking strategies offers an in-depth insight into the ranking of small molecules interacting with the AHR protein, paving the way for novel avenues in drug discovery across various medical domains.

[O7] The AhR is a Dominant Mediator of IFNγ Signaling Leading to Immune Checkpoint Up-regulation and Survival of Oral and Head and Neck Carcinomas

Megan Snyder [1], Zhongyan Wang [2], Brian Lara [2], Jessica Kenison-White [3], Vinay Kumar Duggineni [4], Gang Song [2], Jocelyn Fimbres [2], Mohammed Muzamil Khan [5], Stefano Monti [4,5], and David H. Sherr[2]

[1]Graduate Program in Genetics and Genomics, Boston University Chobanian & Avedisian School of Medicine
[2]Department of Environmental Health, Boston University School of Public Health
[3]Brigham and Women's Hospital, Harvard Medical School
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While immunotherapy with immune checkpoint-targeted antibodies has shown efficacy in several cancers including oral squamous cell (OSCC) and non-small cell lung (NSCLC) cancers, not all patients respond and not all of those who respond achieve durable remission. One limitation in improving immune immunotherapy outcomes is the lack of a complete understanding of factors regulating immune checkpoints. While the AhR and its environmental ligands have long been associated with immunosuppression, the AhR's role specifically in regulating immune checkpoints has not been fully studied. Here, we identified an interaction between the AhR and two immune checkpoints, PD-L1 and IDO, in the context of OSCC and NSCLC. Specifically, we linked the AhR to a counter-intuitive, IFNγ-mediated pathway leading to PD-L1 and IDO up-regulation. We exploited *AhR*, *CD274* (*PD-L1)*, and *IFNγR* gene-edited murine and human cell lines, OSCC and NSCLC mouse models, and bulk- and single cell-RNA sequencing of malignant cells and tumor-infiltrating leukocytes. The data demonstrate: **1)** While not affecting cell growth *per se*, AhR deletion from OSCC MOC1 cells and NSCLC CMT167 cells results in no OSCC growth and significantly diminished NSCLC growth *in vivo*. **2)** In both cases, mice that fail to generate tumors on transplant with AhR-knockout (AhR-KO) cells are either completely (OSCC) or partially (NSCLC) immune to further challenge with wildtype tumors. **3)** Immunity is related to AhR control of an IFNγ-driven pathway that up-regulates IDO1 and PD-L1, **4)** AhR-driven IDO expression (via IFNγ dependent or independent pathways) in malignant cells results in excess Kynurenine (Kyn), a known AhR ligand and precursor to other tryptophan-derived AhR ligands, in an AhR→IDO→Kyn→AhR amplification loop, **5)** AhR control of IFNγ-induced IDO and PD-L1 appears to occur through AhR modulation of JAK/STAT signaling and *IDO1* or *CD274* mRNA sequestration by a novel long non-coding RNA, and **6)** Tumor immunity induced with AhR-KO OSCC and NSCLC cells occurs concomitant with a shift in several immune subsets, most notably an increase in what genomically appear to be immunocompetent $CD4^+$ and $CD8^+$ T cells and/or neutrophils. Finally, we note that, while AhR inhibitors should be able to target both the malignant cells and AhR^+ immunosuppressive tumor-infiltrating cells and therefore be at least as effective as AhR knockout within just the malignant cells, they never are. Here we provide a hypothesis for this limitation.

[O8] Role of the AHR pathway in melanoma pathogenesis and treatment

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Melanoma, the most lethal type of skin cancer, accounts for almost 2% of cancer cases worldwide. Its incidence has steadily risen over the last decades mainly due to increased recreational UV exposure. In recent years, evidence is accumulating that the aryl hydrocarbon receptor (AHR) functionally participates in melanoma pathogenesis. UVB irradiation induces the production of the tryptophan metabolite 6-formylindolo(3,2-b)carbazole (FICZ), a high affinity AHR ligand that stimulates pigment production in the skin. The AHR-dependent tanning response provides protection against the development of primary melanomas. However, once melanocytes have undergone malignant transformation, activation of the AHR supports the emergence of melanoma cell subpopulations that are able to migrate, grow invasively and metastasize. This process is facilitated by an inflammatory microenvironment where AHR activation in melanoma cells drives the acquisition of dedifferentiated phenotypes and AHR activation in immune cells impairs anti-tumoral defenses. Importantly, the expression of AHR in dedifferentiated melanoma cell subpopulations also mediates the resistance against novel therapies that target BRAF and MEK to inhibit the MAPK signaling transduction cascade. Furthermore, activation of the tryptophan-kynurenine-AHR pathway modulates the efficacy of novel immunotherapies that target T cells with antibodies blocking immunoregulatory receptors such as PD-1. In my presentation, I will review the complex and multifaceted role of the AHR pathway in the pathogenesis and treatment of melanoma, highlighting current avenues of investigation and potential strategies for therapeutic intervention.

[O9] AhR as a regulator of innate and adaptive immunity in the tumor microenvironment

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The microbiome can significantly impact cancer responses to therapy and overall survivorship. In particular, increased microbial diversity and the presence of specific bacterial taxa in the microbiome community strongly correlates with improved responses to immune therapy. However, defining mechanisms of microbiome-immune system crosstalk and its influence on therapy is an ongoing challenge. In this seminar I will describe our recent work identifying a mechanism of microbial metabolite/immune system communication and its role in driving immune-suppressive programs in intratumoral macrophages and T cells by activation of the aryl hydrocarbon receptor (AhR). I will also discuss recent developments in the field that reveal the challenging complexity of bacterial tryptophan metabolization, disease pathology, AhR function, and the response to a variety of therapeutic modalities.

[O10] Aryl hydrocarbon receptor promotes Alzheimer's disease development and progression through microglial modulation in the APP^{NL-F} knock-in mouse model

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An interaction between intrinsic and environmental factors probably contributes to the molecular processes that drive Alzheimer´s Disease (AD). Although variation in specific genes increases the risk of AD, one of the main risk factors is age. However, how molecular processes of aging predispose to, or become deregulated in AD, still remains to be understood. Studies in different organisms from invertebrates to humans show that the Aryl Hydrocarbon Receptor (AhR), that integrates environmental stimuli (from pollutant to diet components with agonist properties) into transcriptional changes, is implicated in the aging process. Therefore, we decided to investigate the role of AhR in the development of age-associated neurodegeneration. To address this issue, we characterized the effect of AhR deletion and activation by specific agonists in the APP^{NL-F} knock-in mouse model of AD. First, we found that the expression of AhR increases in the APP^{NL-F} mouse with age and that this increase is mostly associated with microglia and astrocytes. Importantly, our data also demonstrate that AhR plays a deleterious role in AD since AhR activation by the specific AhR agonist indoxil sulphate (I3S) exacerbates amyloid plaque formation and plaque-associated dystrophic neurites while AhR deletion reduces, on the contrary, the amyloid phenotype and the associated neurodegeneration. In addition, our data point toward microglia as a possible mechanism by which AhR modulates the development of EA. In fact, we found that both homeostatic (or non-plaque-associated) and plaque-associated microglia undergo qualitative and quantitative changes in the absence of the AhR, that could explain, at least in part, the neuroprotective effect that is observed when AhR is removed.
Overall, our data suggest that AhR plays a pivotal role in the development and progression of AD and that the AhR pathway and/or its modulation by exogenous or endogenous agonists can be explored for AD therapy.

[O11] Tonic activation of Aryl Hydrocarbon Receptor by food-derived ligands is essential for the efficacy of immune checkpoint blockade therapy

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Cancer immuno-surveillance and response to checkpoint blockade therapy can be affected by multiple environmental factors, including nutrition. However, the direct effects of individual nutrients on anti-tumoral immune responses remain poorly understood. Here we addressed the impact of dietary ligands of Aryl Hydrocarbon receptor (AhR), a transcription factor activated by indoles and tryptophan catabolites generated through food digestion and microbiota metabolism. To this aim, we analyzed pre-clinical tumor models in mice fed on a diet naturally poor in AhR ligands, or the same diet enriched for Indole-3-carbinol, a phytonutrient cleaved by stomach enzymes into AhR agonists. We found that diet-derived AhR ligands were essential for the efficacy of anti-PD1 therapy. By using mice harboring microbiota deficient for the production of AhR ligands, we further showed that microbiota-derived AhR ligands were dispensable for anti-PD1 therapy. Lack of dietary AhR ligands did not significantly affect the baseline immune landscape, but impaired the infiltration of NK cells upon anti-PD1 treatment. Using a series of mice deficient for AhR in specific immune cells, we showed that T cells, but not NK cells or myeloid cells, are the direct cellular targets of dietary AhR ligands. Transcriptomic analysis showed that tonic activation of AhR promotes anti-PD1-mediated reinvigoration of progenitor exhausted CD8 T cells by controlling their metabolic fitness, and licences the functional program of effector CD8 T cells including secretion of NK cells chemo-attractants. Our work allows a better understanding of the role of dietary nutrients in anti-tumor immune responses and will have important implications for the rational design of dietary interventions for improving the efficacy of checkpoint blockade therapy.

[O12] Exploration of the pathophysiological role of aryl hydrocarbon receptor in Langerhans cells at epicutaneous protein sensitization and psoriasis

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Atopic dermatitis (AD) is a common allergic disease with significant interactions among genetic susceptibility and environmental exposures. I reported lifetime smoking exposure increases the risk of adult-onset AD. Epicutaneous protein sensitization is pivotal to regulate the immune abnormality in AD. The aryl hydrocarbon receptor (AhR) acts an environmental sensor regulating immune responses. In the skin, AhR is expressed in several cell types, including keratinocytes, epidermal Langerhans cells (LC), and dermal dendritic cells (DC). We reported that AhR activation by benzopyrenes (BP), a major PAH in smoke, is accentuated in AD skin. In mice, BP increases LC migration in vivo, and increases Th2 cytokines during in vitro challenge. The increased cytokines were reduced in the AhR defected mice. However, how AhR activates or inhibits cutaneous immune responses remain controversial, due to differential cell-specific functions and activation ligands of AhR. Therefore, we sought to investigate the role of AhR in LC in the skin. We generated Langerin-specific mice lacking AhR and then tested them in an epicutaneous protein (ovalbumin, Ova) sensitization model. Immunofluorescence microscopy and flow cytometry revealed that Langerin-AhR$^{-/-}$ mice harboured fewer LC with fewer and stunted dendrites in the epidermis as well as fewer LC in skin-draining lymph nodes (LN). Moreover, in the absence of AhR, we detected an enhanced Th2 (increased IL-5 and IL-13) and Tr1 (IL-10) response when LN cells were challenged with Ova *in vitro*, though the number of regulatory T cells (Treg) in the LN remained comparable. Langerin-AhR$^{-/-}$ mice also exhibited increased blood levels of Ova-specific IgE. Taken together, deletion of AhR in langerin-expressing cells diminishes the number and activation of LC, while enhancing Th2 and Tr1 responses upon epicutaneous protein sensitization. Then, since smoking is also known to worsen psoriasis, another inflammatory skin disease with Th17 and Th1 polarization, I will show preliminary results showing the decreased skin thickening and decreased lymph node LC in imiquimod-induced psoriasis in Langerin-AhR$^{-/-}$ mice and how autophagy might regulate them.

[O13] Host and microbiota derived AhR agonist: role in intestinal homeostasis and IBD

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The gut microbiota is a crucial actor in human physiology. Many of these effects are mediated by metabolites that are either produced by the microbes or derived from the transformation of environmental or host molecules. Tryptophan (Trp) metabolites are among the most studied categories of metabolites involved in host-microbiota interactions.
In the gut, the three major tryptophan metabolism pathways leading to serotonin, kynurenine and indole derivatives are under the direct or indirect control of the microbiota and are tightly interconnected. Beyond changes in microbiota composition, several key functions are altered in disease and this play a role in disease onset, chronicity or complication. We showed that the ability of the gut microbiota to produce AhR agonists from tryptophan metabolism, as well as other trp metabolism pathways are impaired in inflammatory bowel disease. More importantly, correcting these functional defects, either pharmacologically or through the administration of bacteria that are natural producers of AhR agonists, induces beneficial effects. Manipulating the tryptophan metabolism could be of therapeutic interest for many human diseases.

[O14] The anti-inflammatory topical medication tapinarof modulates mitochondrial metabolism and nucleic acid release

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Tapinarof is a novel anti-inflammatory, topically applied AHR agonist that is effective in the treatment of both psoriasis and atopic dermatitis. Psoriatic patients treated with Tapinarof have long-lasting (average four month) remissions after discontinuing the medication. Although this medication is currently in clinical use, there have been no prior mechanistic studies in living human skin. We tested topical tapinarof in NSG mice grafted with human skin and injected with allogeneic PBMC; this model generates a human T cell mediated inflammatory dermatitis in the human skin graft. Grafts treated for three weeks with vehicle control or topical tapinarof were analyzed by bulk RNA sequencing and multiplex immunostaining. Tapinarof reduced Type 1, 2 and 17 mediated T cell inflammation and inhibited macrophage, DC and NK activation. Cytoplasmic mtDNA and mtRNA induced nucleic acid sensing, reduced type I, II, and III IFN production, CGAS/STING, and inflammasome activation were also impaired. Psoriatic 3D human keratinocyte and fibroblast constructs treated in vitro with Tapinarof demonstrated profound metabolic impairments and energy starvation via inhibition of hypoxia sensing, glycolysis, fatty acid β-oxidation, and glutamate influx. In response to this low energy state, cells halted mRNA splicing and protein production. Tapinarof-treated human T cells also showed inhibition of glycolysis and reduced effector functions. In summary, tapinarof likely exerts immunomodulatory effects by acting at the mitochondrial level, reducing mitochondrial mtDNA and mtRNA release, thereby reducing inflammatory tone, and inducing metabolic constraints by blocking the ability of cells to use glycolysis to generate energy in the hypoxic skin microenvironment.

[O15] Endothelial sensing of Aryl Hydrocarbon Receptor ligands regulates intestinal barrier immunity

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Endothelial cells (ECs) that line the blood and lymphatic vasculature display tissue-specific functional adaptations to support organ homeostasis. In the small intestine, the blood vasculature must facilitate nutrient transport, while maintaining a barrier against gut microbes; whereas lymphatic vessels coordinate lipid transport and enteric immune cell trafficking. AHR-mediated environmental sensing has shown to be tissue-protective in intestinal epithelial and immune populations, but whether enteric ECs actively sense AHR ligands remains to be elucidated. Here, we demonstrate that AHR acts as a critical node for EC sensing of dietary metabolites in the gut, transducing environmental signals into pro-quiescent and anti-inflammatory responses. First, we generated a comprehensive single-cell endothelial atlas of the mouse small intestine, uncovering the cellular complexity and functional heterogeneity of blood and lymphatic ECs at the gene, pathway, and gene regulatory network level. Next, we mapped responses to AHR ligands onto this heterogeneity, demonstrating both a universal sensitivity of enteric ECs to AHR ligands, and associated AHR ligand-induced tissue-protective transcriptional signatures promoting cellular quiescence. Similarly, in human ECs, AHR signalling promoted quiescence and restrained activation by inflammatory mediators. Importantly, endothelial AHR-deficiency in mice led to dysregulated inflammatory responses, initiation of proliferative and angiogenic pathways, and heightened susceptibility to infection with enteric pathogen Yersinia pseudotuberculosis. Together, our data demonstrate that the integration of dietary cues via endothelial AHR signalling is a key to maintaining intestinal homeostasis through the promotion of EC quiescence and vascular normalcy.

[O16] Aryl hydrocarbon receptor ligands from maternal microbiota shape skin barrier development.

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The skin is the body's outermost organ and is constantly exposed to environmental threats. It also accounts for 30% of body weight at birth. Defects in skin development early in life are associated with an increased risk of infection and allergy. As we have previously shown that aryl hydrocarbon receptor (AHR) ligands from the maternal microbiota reach the fetus and that AHR deficiency is associated with impaired intestinal barrier formation (*Gomez de Agüero et al. Science 2016*), we investigated the contribution of maternal microbial-derived metabolites to establish the cutaneous barrier by birth.
We used a sophisticated gnotobiotic murine model based on an auxotrophic *Escherichia coli* strain for controlled gestational colonisation of pregnant germ-free dams with sterile offspring exposed to maternal microbial metabolites during embryonic development (Gomez de Agüero et al. Science 2016). Single-cell sequencing, flow cytometry and microscopic analysis were used to investigate the effect of maternal microbial metabolites on the development of the epidermis, the outer layer of the skin that concentrates most of the barrier elements needed to protect the organism. Only the epidermis of the offspring of gestationally colonised dams showed a developed architecture consisting of several distinct and functional interfollicular stratums and properly formatted hair follicles. Pre-gestation or postnatal exposure to microbial metabolites could not compensate for their absence during gestation. Using ^{13}C-labelled bacteria, mass spectrometry analysis and 3D epidermal organoid models, we demonstrated that AHR ligands from the maternal microbiota modulate the stemness of keratinocyte stem cells. Indeed, only these trained keratinocyte stem cells are superior to expand and differentiate in order to regenerate the defective epidermal architecture following neonatal injury. Furthermore, only the offspring of gestationally colonised dams has a functional perinatal permeability barrier as evidenced by preventing abnormal transepidermal water loss and chemical penetration. In conclusion, our results show that AHR ligands from maternal microbiota shape embryonic epidermal development and establish the skin barrier by birth.

[O17] Targeting the aryl hydrocarbon receptor (AHR) with the selective inhibitor BAY 2416964 to revert cancer-associated immunosuppression

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Immunotherapeutic approaches for cancer treatment have become an integral part of standard therapy in multiple tumor types. Inhibiting the immune checkpoint PD-1 has resulted in significant survival benefit for patients with various cancers, however, the majority of patients still experience therapy resistance. Preclinical data suggest that activation of the aryl hydrocarbon receptor (AhR) pathway by ligands predominantly accumulated in the tumor microenvironment acts immunosuppressive, and its inhibition represents a promising attack point to unleash anti-tumor immunity. Here we report the identification of BAY 2416964 a potent and highly selective small molecule AhR antagonist. Comprehensive preclinical evaluation revealed that BAY 2416964 blocks ligand-induced activation of AhR and counteracts its immunosuppressive effects by reversing anti-inflammatory phenotype of monocytes, increasing dendritic cell-mediated but also direct T cell activation. Treatment with BAY 2416964 reduced the number of inhibitory immune cells (MDSC, Tregs) in preclinical cancer models and improved the effectiveness of PD-1 blockade. With its proinflammatory properties, the AhR inhibitor BAY 2416964 represents a potential treatment option in tumors with AhR-dependent immunosuppression.

[O18] Allosteric targeting of the AhR as a novel pharmacotherapeutic strategy

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Pharmacological targeting of the AhR is an emerging strategy in the therapy of various diseases, such as atopic dermatitis, acne, inflammatory bowel disease, cancer etc. The potential therapeutics stem from targeted (rational design, repurposing AhR-active drugs) and untargeted (high-throughput screening, serendipity) approaches. All existing AhR-active drugs are small molecules, which act as AhR orthosteric ligands, comprising both agonists and antagonists. The recent trends in receptors targeting are in favor of allosteric ligands, due to their multiple pharmacological advantages. The conception of allosteric modulation of the AhR has not been pursued so far. Here we report monoterpenoid *S*-carvone as a negative allosteric modulator (NAM) of the AhR, which antagonized human AhR, activated by xenobiotic, endogenous, and microbial ligands (1). *S*-carvone acted by the atypical mechanism, whereas it did not inhibit ligand-dependent AhR nuclear translocation, it blocked dimerization of the AhR with ARNT, and all downstream mechanistic events. *S*-carvone bound the AhR allosterically, at a site within bHLH and PAS-A domains. The mutation analyses revealed about the key role of Tyr76 in the interaction between *S*-carvone and AhR. Oxygen moiety in the *S*-carvone molecule was essential for binding to the AhR, because de-oxy-*S*-carvone (*D*-limonene) did not bind AhR. We showed that *S*-carvone antagonizes AhR also *in vivo*, in mice ears topically treated with BaP. *S*-carvone reversed the augmentation of chemokine *mCXCL5* levels by BaP in UV-irradiated mice ears, which is of potential clinical importance, because CXCL5 was identified as a culprit of UV irradiation-induced inflammatory pain in sunburned subjects. In conclusion, a discovery of dietary monoterpenoids as AhR NAMs opened a new avenue in therapeutic targeting of the AhR by exploiting functional allosteric interactions between AhR and small molecules.

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[O19] Identification and in vitro validation of new AHR ligands as potential therapeutics for inflammatory bowel diseases

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Disruption of the intestinal barrier has been shown to contribute to the development of inflammatory bowel disease (IBD). The aryl hydrocarbon receptor (AHR) plays a critical role as regulator of inflammatory processes at epithelial barriers in the gut, lung, and skin. Therefore, the AHR is considered as a therapeutic target in chronic inflammatory diseases at epithelial barriers. Although a plethora of endogenous and exogenous AHR ligands has been reported in the literature, many of them are inefficient or exert toxic effects or their PD/PK and toxicological profile is still unknown. Thus, there is an urgent medical need of pharmacologically and toxicologically well-characterized AHR ligands as promising new therapeutics for IBD. The aim of this study was to identify and validate safe and efficient AHR agonists that positively modulate inflammatory events in the intestine as potentially new drugs for IBD. Using a luciferase reporter gene assay in a high-throughput screening format, 90 candidates activating the xenobiotic response element through AHR ligation and nuclear translocation were identified from a total of 7,448 compounds. As selection criteria in the second round, we defined the absence of cytotoxic effects and the induction of interleukin (IL)-10, as anti-inflammatory key player in IBD, with concomitant suppression of IL-1β in murine macrophages. In addition, there should be no significant CYP1A1 induction in Caco-2 cells used as an *in vitro* model for the intestinal epithelium. Fifteen promising compounds from this second selection round were evaluated in terms of compound class, effective dose, and clinical evaluation status for other indications. Five out of these 15 compounds were selected and analyzed *in silico* for their molecular interactions with AhR and *in vitro* for their efficacy and toxicity in organoid models derived from primary intestinal epithelial cells of IBD patients. Three of these compounds showed barrier-restoring as well as immunomodulating effects, such as an increase of the transepithelial electrical resistance, the induction of tight-junction proteins (i.e., ZO-1, CLDN1, and OCLN) or the reduction of TLR4 expression, moderate increase in IL-10R and IL-22R expression and induction of antimicrobial peptides (i.e., Reg3A), respectively. After evaluation of all *in vitro* and ongoing *in vivo* studies, one compound was selected for a confirmatory preclinical study using the chronic dextran sulfate sodium colitis model.

[O20] Antibiotic Modulation of the Gut Microbiome Ameliorates Hypertensive Organ Damage

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Background: High blood pressure is associated with measurable inflammation, which precedes and promotes damage to the heart and kidneys. Microbiome-derived metabolites, particularly indole metabolites, modulate inflammation and host physiology, partially through AhR signaling. To better understand the potential of a microbiome-targeting therapeutic approach for organ protection in hypertension, we used narrow-spectrum antibiotics without enteral absorption to deplete gram-negative or -positive bacteria in double transgenic rats (dTGR).

Methods: Four-week-old dTGR (transgenic for human renin and angiotensinogen) were treated with oral Vancomycin (Vanco), Polymyxin B (Poly) or Vehicle (Veh) for 3 weeks. Seven-week-old SD rats were included as healthy controls. Microbiome, clinical and immune phenotype were analyzed by shotgun metagenomic sequencing, echocardiography, telemetric blood pressure (BP) measurement, clinical chemistry, bulk RNAseq and flow cytometry.

Results: Hypertensive kidney damage was ameliorated in Vanco treated dTGR, as assessed by renal *Lcn2* expression, blood urea nitrogen and albuminuria. Vanco treated dTGR had significantly decreased cardiac hypertrophy. Poly treatment showed no effect. BP levels for both antibiotic treatments were not significantly different from Veh, despite a significantly improved endothelium-dependent and –independent vasorelaxation in isolated mesenteric arteries in both treated groups. Bulk RNAseq of heart and kidney showed alterations to AhR target genes in Vanco-treated dTGR. Surprisingly, Vanco treatment led to a massive increase of gram-positive *Lactobacillus murinus* and associated gene abundance for the production of indole lactic acid (ILA, via araT) We could previously show that ILA inhibits differentiation of pathological Th17 cells through AhR modulation. In line, Vanco treatment could reduce the increased number of pathological Th17 in the kidney and intestine of dTGR.

Conclusion: Modulation of the intestinal microbiome by narrow-spectrum antibiotics affects hypertensive organ damage. Our data underscores the importance of the gut microbiome in modulating hypertensive organ damage and helps to identify potential therapeutic strategies in the microbiome. Ongoing experiments investigate pro- and postbiotic therapeutic approaches and their AhR dependency.

[O21] The role of Aryl hydrocarbon Receptor and Polycyclic Aromatic Hydrocarbons in Combustion Particle Toxicity

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Air pollution is the 4th leading risk factor driving the global burden of disease, causing 6.67 million premature deaths in 2019. Particulate matter (PM) from combustion sources such as traffic, industry, and domestic heating are considered central for the adverse effects of air pollution. Understanding the mechanisms of combustion particle toxicity may shed light on the components and sources of outdoor air PM driving adverse health effects. This may enable more targeted and efficient policies to improve air quality and promote health and wellbeing, beyond current regulations of total PM mass.
The aryl hydrocarbon receptor (AhR) is a ligand dependent transcription factor, originally discovered for its ability to bind polycyclic aromatic hydrocarbons (PAHs) and dioxin-like compound and regulate PAH metabolism. It is now clear that AhR regulates a variety of biological and toxicological responses, overlapping to a considerable degree with the effects of combustion particles and ambient air PM. Studies from our lab and others also suggest that AhR activation is among the most sensitive responses of combustion PM exposure, induced at orders of magnitude lower concentrations than many other endpoints. This talk will discuss the potential role of the AhR and PAHs in combustion PM toxicity based on previous and ongoing work, with a main focus on intracellular signalling and regulation of inflammatory reactions in lung epithelial cells and vascular endothelial cells. Lipophilic organic chemicals may account for much of the cellular effects induced by combustion PM and stimulate both genomic and non-genomic AhR signalling. These effects may not be restricted to classical AhR activating PAHs such as benzo[*a*]pyrene, but likely also involves lower-molecular weight species including pyrene and phenanthrene.

[O22] A dynamic Aryl hydrocarbon Receptor (AhR) as a sensor of environmental pollutants

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The AhR was discovered in the 1970s as a sensor of exogenous chemicals such as dioxins and Polycyclic Aromatic Hydrocarbons (PAHs) inducing cytochrome (CYP)450 family 1 proteins and their enzyme activity. The AhR dimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT) another PER-ARNT-SIM (PAS) domain containing protein to regulate genes of the AhR gene battery including CYP4501A1 and CYP1A2 through the canonical AhR pathway. Several decades later it became evident that the AhR is involved in inflammation and the expression of genes regulating immune responses and that the AhR is involved in other signaling pathways such as Toll-like receptors (TLR) and Nuclear factor-κB (NF-κB). More recently, studies have shown that the AhR responds to the exposure of ambient particulate matter (PM) air pollution and mediates at least partially the toxicity of PM. PM from various sources including traffic, engine emissions, industrial activities, and wildfire smoke contain PAHs and dioxin-like chemicals which bind to and activate AhR. Exposure to PM generated via traffic related air pollution or wildfires involves complex mixtures of chemicals activating AhR signaling and other signaling proteins of non-canonical AhR pathways. Exposure to PM is well known to be associated with chronic inflammatory diseases including asthma, atherogenesis and cardiovascular disease as well as an increased risk to develop certain types of cancer. Although, the AhR is involved in developmental and many biological pathways, the chronic and sustained activation of AhR by environmental pollutants and chemical mixtures can lead to various pathological endpoints and adverse health effects. The impact of exposure to PM from various sources on the expression and dysregulation of critical factors involved in atherogenesis and carcinogenesis will be discussed.

[O23] AhR and oxidative stress reporter mice as a tool for understanding transcriptional responses to Aroclor 1254 exposure across multiple generations.

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Environmental exposure to pollutants can cause or exacerbate human disease. Polychlorinated biphenyls (PCBs) are environmental pollutants that remain ubiquitous in the ecosystem due to their resistance to biological degradation. Exposure to PCBs is associated with cancer and neurotoxicity in humans. The toxic effects of PCBs are mediated through various mechanisms involving binding to, and activation of, nuclear receptors, including the aryl hydrocarbon receptor (AhR). It has been proposed that other mechanisms of toxicity may also be involved, such as oxidative stress due to the production of reactive oxygen species. However, studying the interplay of transcriptional responses to PCB exposure *in vivo* has not been possible due to a lack of reliable biomarkers. As part of a large UKRI-funded multidisciplinary consortium, we utilise stress reporter mouse models to define the mechanisms of toxicity of environmental agents, including complex mixtures. Using Aroclor 1254 as a model compound, we show that exposure to a PCB complex mixture induces activation of both the Cyp1a1 (AhR pathway) and Hmox-1 (Nrf2 and inflammation) reporters in mouse tissues, most notably in the liver. By crossing the Hmox-1 reporter into a Nrf2-null background, we found Aroclor 1254-induced Hmox-1 expression to be largely Nrf2 independent. Strikingly, we demonstrate the utility of the Hmox1 reporter for tracking the consequences of Aroclor 1254 exposure across multiple generations, as transmitted through trans-lactational exposure. We also report a full RNA-seq analysis of livers from control and Aroclor 1254-exposed reporter mice. We propose our reporter mouse models have great potential to aid in the understanding of the transcriptional effects of toxicological exposures, how these exposures may impact on human health and disease, and can contribute to the knowledge of the long-term toxicological consequences of exposure to environmental chemicals and complex mixtures. This work was supported by UKRI grant number NE/W002213/1.

[O24] AhR-Mediated Impacts of BaP-Coated CeO_2 Nanoparticles on the Human Placental Barrier

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The human placenta, a transient and vital organ for fetal development, may undergo functional disruptions caused by xenobiotics present in maternal blood. Epidemiological studies have established a link between maternal exposure to air pollution and adverse pregnancy outcomes, such as premature birth and low birth weight. Emerging pollutants, as cerium dioxide nanoparticles (CeO_2 NP), prompted the OECD in 2010 to prioritize their study due to insufficient knowledge concerning their impact on human health. Since the 2000s, they have been used as additives in diesel fuels and cigarettes due to their catalytic properties, and thus released into the air. Human exposure to CeO_2 NP is in combination with other pollutants from common emission sources, including polycyclic aromatic hydrocarbons (PAH). Benzo-a-pyrene (BaP) stands as a prototype of PAH, known for its carcinogenic, mutagenic, reprotoxic, and endocrine-disrupting properties. Moreover, BaP emissions are increased by 35% with the use of CeO_2 NP in fuels. Therefore, mixtures of CeO_2 NP and BaP better reflect environmental exposures. Subsequently, we produced BaP-coated CeO_2 NP at two ratios: one resembling the PAH/ultrafine particles ratio found in Parisian air and another enabling BaP to cover the CeO_2 NP surface. Subsequently, we investigated the biological impacts of these BaP-coated CeO_2 NP on chorionic villi and purified villous cytotrophoblasts (VCT) from human placentas at term. Confocal and transmission electron microscopy confirmed the internalization of pollutants in trophoblasts. By using XRE-plasmids, we evaluated AhR-activation involved in BaP-bioactivation and the downstream induced stress signaling pathways (p53, p21, γH2AX) by RT-qPCR and Western blot. While there was no significant difference in cell toxicity, exposed VCT displayed notable variations in AhR activity and in critical trophoblasts functions such as the differentiation capacity to form the syncytium when exposed to BaP-coated CeO_2 NP as compared to co-exposures or individual exposures. The use of an AhR antagonist revealed that the stress pathways induced by BaP in VCT were driven by the activation of the AhR, and co-exposure with CeO_2 NP was observed to mitigate this effect. These findings highlight the modulated effects of BaP when stably coated on CeO_2 NP, potentially modifying its metabolization kinetics and thus its biopersistence, giving a closer reflection of the environmental reality.

[O25] Exposure to airborne particulate matter increases the risk for atopic dermatitis: Clues for an involvement of AHR-dependent NADPH-dependent oxidoreductases.

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The pathogenesis of atopic dermatitis (AD) involves an impairment of the skin barrier by an interplay of genetic and environmental factors. There is increasing evidence that a cutaneous exposure to airborne particulate matter (PM) contributes to the development of AD. A large part of the adverse effects induced by PM exposure is attributed to polycyclic aromatic hydrocarbons (PAHs), which are bound to the surface of combustion-derived PM. *Per se* non-toxic, PAHs are metabolically activated resulting in the formation of bulky DNA adducts, the generation of reactive oxygen species (ROS) and oxidative damage. In keratinocytes, PAH-induced ROS is mainly generated by oxidoreductases, which continuously reduce and oxidize CYP1-derived PAH-diols, thereby producing superoxide and hydrogen peroxide. Besides NADPH:quinone oxidoreductase 1, the NADPH-dependent oxidoreductases aldo-keto reductase 1C3 (AKR1C3) and carbonyl reductase 3 (CBR3) catalyze this redox-cycling of PAH metabolites. The resulting oxidative damage to epidermal cells probably impairs skin barrier integrity and function and thus contributes to AD development. In addition, AKR1C3 may promote AD-related inflammatory responses by reducing mast cell-derived prostaglandin D_2 to the Th2-stimulatory metabolite 9α,11β-prostaglandin F_2.

We found that a treatment of human keratinocytes with the PAHs benzo[a]pyrene and benzo[k]fluoranthene as well as with extracts of traffic-related PM resulted in an upregulation of AKR1C3 and CBR3 *via* a non-canonical AHR signaling pathway, involving the sequential activation of c-Src, EGF receptor and NRF2. Importantly, a repetitive topical application of PAH-rich PM, i.e. diesel exhaust particles, confirmed an upregulation of AKR1C3 and CBR3 in human skin *ex vivo*. To assess the clinical relevance of both oxidoreductase-encoding genes for AD, we analyzed data from the German GINIplus/LISA birth cohort study, enrolling 457 participants with available AD diagnosis at the 15-year follow-up examination, air pollution exposure data and genetic data. The study revealed that under constant chronic PM exposure a gain-of-function single nucleotide polymorphism (SNP) in the AKR1C3 gene (rs12529), increases the risk for developing AD by 38 % *per* one effect allele. While we did not identify a specific SNP in the CBR3 gene which modulates AD risk, a risk score consisting of 52 SNPs was associated under constant chronic PM exposure with AD risk, which strongly pointed to an involvement of CBR3 in AD.

Our data indicate that alterations in the expression and activity of AKR1C3 and CBR3, whether due to genetic or environmental factors, may critically affect the development and worsening of AD by airborne PAH/PM exposure. In conclusion, the data emphasize the important role of AHR as a regulator of oxidative and inflammatory stress responses in air pollution-exposed skin, and identify this protein as a suitable target for chemopreventive measures.

[O26] AHR and regulation of antiviral immunity

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In spite of numerous advances in medicine and public health, viral infections remain a major source of global morbidity and mortality. Yet, we do not understand why, during a viral outbreak, some individuals become extremely ill, while others do not. Recent studies of the aryl hydrocarbon receptor (AHR) provide clues about how small molecules from the environment influence immune responses to infection. In human population studies, pollutants that bind AHR are associated with poorer responses to immunizations and increased incidence or severity of infection. This association is particularly notable for exposures during early life development. Building off these studies, we are using mouse models to delineate cellular level root causes of AHR-mediated variation in antiviral immune defenses, using common human viral pathogens, such as influenza A virus (IAV). AHR activation during development significantly alters T cell clonal expansion and differentiation upon infection with IAV. This is particularly notable because developmentally exposed but immunologically naïve animals had no apparent differences in lymphoid organ cellularity or the distribution of immune cell populations. Thus, signals delivered via AHR during development, via maternal treatment with an AHR agonist, lead to cryptic changes in T cells, which are not detectable until the immune system is challenged. Using conditional knockout mice, bone marrow transplantation, and adoptive transfers, we have identified critical changes that are due to direct consequences of AHR signaling in specific types of immune cells, and that other effects are indirect, reflecting alterations to intercellular communication. Given that alterations in T cell responsive capacity are apparent even after the exogenous AHR ligand is no longer present, we hypothesized that AHR activation during development affects epigenetic regulatory machinery, such as DNA methylation patterning. To test this, and delineate causal mechanisms, we combine multidimensional flow cytometry, next generation RNA sequencing, and whole genome assessments of DNA methylation. These approaches, combined with functional studies, have yielded exciting new information about the cellular pathways regulated by early life AHR signaling. One of the mechanisms by which early life AHR signaling produces long durable changes in T cell functional capacity is by altering DNA methylation patterns. This provides important new insight into how AHR signaling affects immune programming, and also affords a new window into approaches to modify T cell responsive capacity later in life.

[O27] Regulation and Function of the AhR in the context of type 2 immunity

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Aryl hydrocarbon receptor signalling has been linked to the regulation of different types of immune responses but not much is known about its physiological role in the context of type 2 immunity. Here, I will present evidence for the role of the AhR in the context of allergic responses and parasite immunity with a particular focus on myeloid cells. Additionally, the regulation of AhR ligand availability by different mechanisms will be discussed as an important layer to regulate AhR activity in the context of type 2 immunity.

[O28] Role of AHR in the control of inflammation

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[O29] AHR limits inflammation in intestinal macrophages

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Environmental triggers play a major role in susceptibility to inflammatory bowel diseases but remain poorly defined. The transcription factor aryl hydrocarbon receptor (AHR) acts as an environmental sensor to ligands derived from external pollutants as well as dietary and microbial metabolites. It plays an important role in promoting barrier integrity in the gut. AHR has also been identified as a risk factor for ulcerative colitis in recent genome-wide association studies. Notably, the site of the genetic association overlaps with a macrophage-specific enhancer that is inactive in intestinal epithelium. However, the role of AHR in intestinal macrophages and how this might contribute to ulcerative colitis pathogenesis remains unknown. We found that AHR is upregulated along the monocyte to macrophage waterfall in the intestine of mice at steady state. To determine what induce AHR in the intestine, we exposed bone marrow-derived macrophages to a range of factors and identified several immune mediators and microbial ligands that upregulated AHR expression. AHR affected the phenotype and composition of macrophage subsets in the colon and regulated the expression of over 300 genes involved in inflammation, apoptotic cell clearance, chemotaxis, response to interferon gamma, cell adhesion and more. Experimental DSS-induced colitis led to downregulation of AHR in macrophages, in particular within tissue-resident populations. Using single cell RNA sequencing, we found that AHR regulated several macrophage subsets during DSS colitis and that lack of AHR resulted in a failure to return to homeostatic conditions. To determine the role of AHR in human macrophages, we established a CRISPR/Cas9 knockout of AHR in primary human monocyte-derived macrophages. Lack of AHR activity was confirmed through loss of expression of canonical AHR target genes upon ligand stimulation. Similar to our findings in the mouse, AHR-deficient human macrophages exhibited a pro-inflammatory phenotype. These results provide first insights into the anti-inflammatory role of AHR in intestinal macrophages and into potential protective mechanisms of AHR in ulcerative colitis.

[O30] Breaking Aryl Hydrocarbon Receptor's Tumor Microenvironment Sensing for Enhanced cDC1-Mediated Antitumor Response

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The immune system plays a pivotal role in governing tumor initiation and growth control. Cancer immunotherapies strive to leverage the immune system for eradicating cancer cells and regulating tumor progression. However, only a small subset of patients exhibit a positive response to immune checkpoint therapy. Conventional dendritic cells (cDCs), including cDC1 and cDC2 subtypes, are crucial for both innate and adaptive immune responses. Notably, cDC1s are pivotal in initiating an anti-tumor response by priming CD8+ T-cells, a function susceptible to environmental signals. The Aryl Hydrocarbon Receptor (AhR), an environmental 'sensor' for specific metabolites, unexpectedly influences tumor evasion mechanisms. Hence, our study evaluated the impact of AhR deletion in cDC1s on the immune response against tumors.

Firstly, through whole-genome analysis, we demonstrated a higher expression of AhR in mature cDC1s compared to cDC2s. Furthermore, AhR deletion in cDC1s significantly enhanced the production of IL-12 and TNF-α. In a cDC1–T CD8+ co-culture model, AhR deletion substantially increased proliferation and IFN-γ production in OTI transgenic T-cells. Intriguingly, single-cell RNA-seq analysis in an *in vivo* fibrosarcoma mouse model demonstrated a high AhR expression in cDC1 tumor infiltrates. Consequently, selective AhR deletion in XCRI expressing cDC1s accelerated the spontaneous immune rejection of a typically progressive fibrosarcoma.

Additionally, to extrapolate our findings to human tumors, we bioinformatically analyzed TCGA sarcoma samples. We found that patients with soft tissue sarcomas (n=231) could be stratified based on AHR gene expression levels, with the AHR^{high} subset exhibiting poorer disease-specific survival (compared to the AHR^{low} subset, p-value<0.05). Further immune contexture analysis of malignant sarcomas indicated that AHR^{high} sarcomas correlated positively with AHR circuitry activity and tumor-tissue cDC1 cell abundance.

Our findings underscore AhR as a metabolic gatekeeper governing the immunoregulatory functions of cDC1s. Additionally, AhR's impact on the prognosis of patients with malignant soft tissue sarcomas suggests its potential as an actionable immune target in this high-risk clinical setting. Overall, these results identify AhR as a novel immune inhibitory target in cDC1s, offering a pharmacological target to counter immune tolerance and resistance to immunotherapy.

[O31] Mice With HIF-1α/ AHR-Double Deficient Keratinocytes Share Similarities With UVB-Exposed Wildtype Littermates

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The transcription factors HIF-1a (Hypoxia-Inducible Factor 1alpha) & AHR (Aryl Hydrocarbon Receptor) act as UVB-responsive sensors in keratinocytes, but are also relevant in other homeostatic and pathologic settings. Both utilize the co-factor ARNT (AHR Nuclear Translocator), but activate distinct target genes modulating cellular metabolism, immune vs. xenobiotic responses and DNA repair. The interplay resp. combined action of HIF-1a & AHR in keratinocytes has hardly been addressed despite potentially high relevance for skin functions. To study this relationship, we bred keratinocyte-specific HIF-1a/ AHR double knock-out mice (DcKO) and generated human single and double-KO (dKO) HaCaT cells for HIF-1a & AhR via the CRISPR-Cas9 method.

The skin of naïve DcKO mice, as compared to WT (wildtype) and single cKO mice, shows features which are expected in UVB-exposed (WT) mice: visible hyperpigmentation of ears and tails mirrored by higher tyrosinase levels, mild acanthosis and significantly less Langerhans cells (LC) with longer dendrite expansion, indicating altered activity compared to controls. Skin-draining lymph nodes revealed increased FoxP3+ Treg frequency and upon *ex vivo* stimulation elevated IL-10 secretion, as seen in UVB-induced immunosuppression. In line, initial single-cell RNAseq analysis of whole skin indicates downregulated signatures for multiple immune processes in the designated LC cluster of DcKO vs. WT mice. Also, cytokine measurements showed differential expression of proteins known to modulate LC function in dKO vs. WT HaCaT cells, indicating the potential of dKO keratinocytes to alter epidermal functions also in human material.

It is unclear whether the DcKO skin phenotype provides improved UVB protection and/ or results from elevated cutaneous stress due to the double protein deficiency in keratinocytes. Initial UVB-induced CPD formation appeared lower in DcKO vs. WT mice, likely due to hyperpigmentation and acanthosis. We will examine the DNA repair capacity in human vs. mouse keratinocytes and mouse skin as well as the functionality of LC and T cell responses in the DcKO mouse. Bulk RNAseq of HaCaT cells will provide additional insight into human relevance. We aim to gain new insight into the interplay of environmental sensors in keratinocytes for the regulation of cutaneous functions.

[O32] Role of the AhR-Repressor (AhRR) in inflammation and metabolism

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The aryl hydrocarbon receptor (AhR) is an important sensor for environmental polyaromatic chemicals and plays an essential role in many immune regulatory processes, both in innate and adaptive immunity as well as in the regulation of physiologic metabolism. To ensure proper regulation of immune control and metabolic changes AhR signalling has to be tightly controlled. AhR activity can be regulated by three AhR-dependent enzymes of the Cytochrome p450 family, CYP1A1, 1A2 and 1B1 by ligand deprivation, and by the AhR-Repressor (AhRR). The AhRR is encoded by an AhR-target gene and competes with the AhR for binding to the cofactor ARNT thereby acting as a negative feedback mechanism to turn down AhR activity. In contrast to the AhR, AhRR expression is largely restricted to immune cells and is upregulated upon AhR activation. Therefore, expression of the AhRR might influence AhR-signaling not only in a cell-type- or tissue-specific manner but may also contribute to context specific influences on AhR activation. We could show that AhRR is involved in the regulation of systemic and local inflammation. Whereas both, AhR and AhRR expression are protective in DSS-induced colitis, AhR signalling, but not AhRR expression, protects from infection with *Salmonella* Typhimurium by restricting overshooting infection-induced extramedullary erythropoiesis and splenic remodelling. Also, AhR signaling contributes to the regulation of body metabolism as both AhR- and AhRR-deficient mice are protected from diet-induced obesity (DIO). To precisely characterize the function of AhR and AhRR directly in adipose tissue and in distinct cell types therein we developed 3D adipose tissue organoids containing macrophages next to adipocytes and precursor cells. Further clarification of the contribution of both AhR and AhRR to these processes will help to understand the function of the AhR/AhRR system in physiology and disease.

[O33] Exploring ligand-dependent interaction dynamics of bHLH-PAS receptors in real time: human AHR and the insect juvenile hormone receptor

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The bHLH-PAS protein aryl hydrocarbon receptor (AHR) depends on specific ligands to heterodimerize with its partner ARNT and to function as a transcription factor. While AHR has been known for almost 50 years and its physiological relevance is immense, the details of its molecular action have emerged more recently owing to resolved structures of various AHR signaling complexes and identification of their physiological ligands. However, elucidating the events preceding and following the ligand binding as they happen inside living cells requires dynamic analyses including real-time monitoring of the signaling complex formation, rather than reliance on end-point assays. Such a dynamic approach is enabled by the split nanoluciferase technology (NanoBiT). We have successfully adopted this tool to explore activity and specificity of signaling by heterodimeric bHLH-PAS protein complexes. Initially, we developed the NanoBiT assay for the juvenile hormone receptor (JHR) of insects, where binding of an agonist stimulates the receptor subunit named Met (methoprene-tolerant) to dimerize with another bHLH-PAS protein taiman (Tai, an ortholog to vertebrate NCoAs) to form a transcriptionally active complex (Tumova and Jindra, 10.1111/febs.16719). Tagging of Met and Tai, or Met and Hsp90, with the nanoluciferase fragments allowed us to monitor in real time the rapid assembly of the Met:Tai JHR heterodimer and the concomitant dissociation of Met from the Hsp90 chaperone complex upon specific agonist binding to Met. This course of events closely resembles the protein interactions during agonist-induced activation of AHR. While Met and AHR are not phylogenetic orthologs, they share similar functional and structural features, particularly within their ligand-binding PAS-B domains. When adapting the NanoBiT technology to study the human AHR signaling complex, we detected rapid, specific and dose-dependent AHR:ARNT interaction upon administration of some AHR agonists (FICZ and indirubin) in living human cells. AHR mutated in its PAS-B domain was incapable of this interaction. We believe that this system may be advantageous relative to previously employed assays for AHR signaling, as it specifically monitors the protein interactions and their dynamics in response to ligand binding.

[O34] AHR Signaling and Cysteine Metabolism in Chronic Intermittent Hypoxia-Induced Hypertension

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Arterial hypertension (HTN) associated with Obstructive Sleep Apnea (OSA) poses a challenge in treatment and needs innovative therapeutic approaches. Our pioneering research linked the AHR, cysteine levels, and elevated blood pressure (BP) associated with OSA's main feature, chronic intermittent hypoxia (CIH). As a ligand-activated transcription factor from the Per-Arnt-Sim (PAS) superfamily, the AHR shares its dimerization partner with the hypoxia-inducible factors (HIFs). We profiled AHR signaling in CIH murine models both before and after the establishment of HTN, with or without AHR blockers. The study has the Ethical approval by DGAV and NMS ethic committee. Briefly, Male Wistar rats (n=5/group) underwent CIH (21-5% O_2, 5.6 cycles/h, 10.5h/day, during the animals' inactive period) for varying durations (1, 7, 14, 21, and 35 days), with normoxic animals serving as controls. To evaluate AHR signaling in CIH-induced HTN, and its crosstalk with oxygen-related signaling (NRF2), animals underwent 21 days of CIH to establish HTN, followed by oral treatment with CH-223191 (5mg/kg/day in vegetable oil) or OHTyr (hydroxytyrosol, 15mg/kg/day in vegetable oil, NRF2 agonist) for 14 days under CIH. Animals under 35 days of normoxia (Nx) or CIH, treated with a vehicle were used as controls. BP was monitored during active and inactive periods using radiotelemetry. AHR-related signaling (*e.g.*, CYP1A1, PON1, HIFs) and cysteine metabolic enzymes (*e.g.*, CSE, CBS) were assessed by Western Blot in metabolic tissues (kidney, liver, skeletal muscle) and quantification of cysteine-related thiols (*e.g.*, GSH, Cys) conducted by HPLC-FD. Transcriptomics analyses of kidney cortex (important role in CIH-HTN), using RNA-Seq. was performed in CH-223191 and OHTyr-treated animals. CIH exposure altered the expression of AHR-related proteins. However, CIH-increased CYP1A1 expression was reversed by both CH-223191 and OHTyr treatment, as the latter also normalized renal levels of oxidized cysteine and CIH-altered cysteine metabolic enzymes. In turn, CH-223191 induced a distinct renal gene expression profile between Nx and CIH conditions, particularly in genes related to heme biosynthesis (ALAD) and solute anion transporters (SLC26A8). Altogether, our findings suggest CIH as an environmental determinant of AHR signaling in a tissue-dependent manner and identify AHR pharmacological pathways, putatively relevant to OSA-HTN.

[O35] Human-specific genetics: new tools to explore the molecular and cellular basis of human evolution

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Our ancestors acquired morphological, cognitive, metabolic, and immunological modifications that enabled humans to colonize diverse habitats, develop extraordinary technologies, and reshape the biosphere. Genome comparisons between humans and the other great apes have identified genetic changes that could underlie our uniquely human phenotypes. In addition, the ability to sequence DNA from ancient bones is providing a historical record of modern human evolution. To model human biology, we are working on next generation stem cell-based systems that recapitulate complex tissue architecture and function in controlled, in vitro environments. We use single-cell technologies (genomics, imaging, computation) to analyze these organoid models and to compare across species. In this lecture, I will discuss our efforts to explore human-specific biology, focusing on the brain and gut.

[O36] AHR sensing microbial infections: a ticket to multiple destinations

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The dynamic interplay between a bacterial pathogen and its host can be conceptualized as an ongoing "arms race," wherein each entity continually adjusts to the evolving strategies of the other. One pivotal mechanism enabling bacterial adaptation to these shifting conditions is Quorum Sensing (QS), a density-dependent cell-to-cell communication system. In pathogenic bacteria, QS orchestrates crucial processes such as biofilm formation and virulence regulation through a cascade of signaling molecules. We postulated that host detection and discrimination of bacterial molecules, including QS molecules, and their expression patterns could facilitate tailored immune responses commensurate with the stage and severity of infection.

Taking advantage of diverse infection models, we unveiled differential modulation of the Aryl Hydrocarbon Receptor (AHR) signaling in infected hosts throughout bacterial infection. This modulation of AHR activity correlates with the relative concentrations of various bacterial molecules, enabling hosts to gauge bacterial community densities and anticipate distinct gene expression profiles and infection kinetics. The AHR, known for its ability to recognize and bind a spectrum of ligands, regulates multiple facets of host defense mechanisms against infection, encompassing ligand degradation, pro-inflammatory mediator expression, immune cell recruitment, and bacterial eradication.

By monitoring infection dynamics, the AHR assumes a pivotal role as a sentinel of infection progression, orchestrating host defenses accordingly. Notably, AHR modulation and activation status exert significant influence on the efficacy of antibiotic therapies, potentially shaping the emergence of antimicrobial resistance and microbial adaptive strategies, namely in the context of Tuberculosis. Our findings underscore the importance of elucidating host-pathogen interactions at the molecular level to inform targeted therapeutic interventions against infectious diseases.

[O37] Environmental influences via AHR on gut barrier regeneration and intestinal infection control

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It has become increasingly clear in the past decades that environmental influences such as diet, lifestyle, or pollution have a substantial impact on physiological health and contribute, together with genetic factors, to shape susceptibility to inflammatory diseases. One of the major molecular entry points for environmental factors is the aryl hydrocarbon receptor (AHR). Inflammatory diseases of the gut are particularly prevalent and mouse models of AHR deficiency have shown that AHR plays important roles in maintaining barrier integrity acting on immune cell types as well as epithelial cells. AHR ligands derived from dietary sources as well as from microbiota metabolites are important for physiological AHR activation. We have shown that dysregulation of AHR activation either by aberrantly high ligand metabolism leading to insufficient AHR activation or by persistent ligands leading to prolonged AHR activation disturb normal functioning of the AHR pathway and result in pathology during infection with pathogens. The large intestine is particularly sensitive to ligand deprivation and infections that target the colon (Citrobacter) cause substantial pathology in the absence of a functioning AHR pathway. This affects immune cells such as ILC3 or Th17 cells which have compromised survival or function (IL-22 production) but also epithelial intrinsic functions such as controlled regeneration and differentiation following infection mediated damage. Infection of the small intestine with the pathogen Cryptosporidium established that AHR activation by dietary indoles safeguards the expansion and function of intraepithelial lymphocytes (IEL) which are the major immune defense against this infection. Cryptosporidium affects particularly immune compromised individuals and infants, and we show that giving a diet deprived of AHR ligands to nursing mice causes increased infection of their newborn offspring, whereas indole supplementation enhances resistance to Cryptosporidium. Altogether the AHR fulfils a crucial role in supporting protection of the intestinal barrier through epithelial intrinsic effects as well as effects on the mucosal immune system.

Keywords: intestinal immunity, tissue regeneration, Citrobacter rodentium, Cryptosporidium, intraepithelial lymphocyte

[O38] AhR-microbiome interactions regulate intestinal IL-10R+ macrophages in non-obese diabetic mice

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The rising incidence of type 1 diabetes (T1D) has been ascribed to environmental factors that act on the gut-pancreas axis. Emerging evidence from epidemiological studies and non-obese diabetic (NOD) mouse models suggests that dietary and microbial exposures in the gut contribute to T1D risk; however, the underlying mechanisms by which these factors influence T1D development are not fully understood. In the gut, macrophages (MACs) sample the luminal environment which informs their subsequent interactions with other intestinal immune cells. We found that in the small intestine, lamina propria MACs are regulated by the aryl hydrocarbon receptor (AhR). We generated AhR knockout (KO) mice on the NOD background and performed scRNAseq on intestinal leukoctyes. AhR KO mice have an increase in a transcriptionally distinct $CD11b^{+}CX3CR1^{+}MHCII^{hi}$ population of $IL\text{-}10R^{+}$ MACs which positively correlate with lamina propria regulatory T cells (Tregs). The ability of these cells to express IL-10R and produce IL-10 is dependent on the microbiome. Both AhR KO and wildtype NOD mouse housed under germ-free conditions have elevated $CD11b^{+}CX3CR1^{+}$ MACs, however these cells do not express IL-10R or produce IL-10. Ex vivo stimulation of $CD11b^{+}CX3CR1^{+}$ MACs from AhR KO mice with LPS rescues IL-10 production. These data suggest that in wildtype NOD mice, AhR regulates the differentiation and/or maintenance of $CD11b^{+}CX3CR1^{+}$ MACS but their functionality depends on microbial signals. Consistent with this two-signal model, we found that AhR KO mice have a significant reduction in T1D incidence when housed under SPF but not germ-free conditions. In contrast, activation of AhR in the intestine by indole-3-carbinol reduced IL-10R+ MACs and promoted T1D development. Thus, both microbial and dietary AhR ligands can regulate $CD11b^{+}CX3CR1^{+}$ MACs in NOD mice. Collectively, modulation of IL10R+ MACs by AhR ligand-sensing is a previously undescribed mechanism through which the gut environment can influence T1D susceptibility.

[O39] The Aryl Hydrocarbon Receptor as novel key driver of lipid metabolism and atherosclerosis

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Cardiovascular diseases (CVD) are the major cause of death worldwide. The main underlying cause of CVD is atherosclerosis. Atherosclerosis is characterized by an imbalanced lipid metabolism and a dysregulated immune response. The aryl hydrocarbon receptor (AhR) is a cytoplasmatic receptor that is highly expressed in the liver and intestine and primarily known for its role in detoxification. However, recent studies suggest that the AhR also has a key role in immune regulation as well as lipid metabolism, suggesting that this receptor can have a major impact on atherosclerosis development.

To investigate this, we generated Apolipoprotein E deficient mice with a full-body knock-out of the AhR (*Apoe*$^{-/-}$*Ahr*$^{-/-}$). These mice were fed a Western-type diet for 12 weeks to induce atherosclerosis development, after which atherosclerotic plaques, plasma and liver lipid levels as well as the number of blood leukocytes were examined. Additionally, the liver was analyzed for cytokine levels and lipid accumulation. RNA sequencing and proteomic analysis were performed on the liver tissue to gain a deeper understanding of affected pathways.

As expected, the inflammation in the liver and the circulating number of blood leukocytes was increased in *Apoe*$^{-/-}$*Ahr*$^{-/-}$ mice compared to *Apoe*$^{-/-}$ controls, assigning a rather anti-inflammatory role to the AhR. Surprisingly however, mice lacking AhR demonstrated highly reduced plaque sizes in comparison to *Apoe*$^{-/-}$ mice. This was in line with strongly reduced plasma cholesterol and triglyceride levels in mice lacking the AhR. The liver cholesterol and triglyceride levels as well as the hepatic lipid deposition showed the same reductive effect, indicating a key role of the AhR in lipid metabolism and hyperlipidemia. RNA sequencing of the liver revealed that particularly lipid-metabolism pathways were impacted in mice lacking the AhR. Furthermore, proteomic analysis uncovered key mediators of the lipid metabolism that are affected by the lack of AhR, like LRP1 and MTTP.

Our study demonstrates a remarkable effect of the AhR on the pathogenesis of atherosclerosis by interfering with lipid metabolism as well as with inflammatory pathways. Since the results are complex and precise mechanisms are not fully understood yet, further research is required. To unravel these likely cell-type-specific mechanisms, more explicit mouse models lacking the AhR in specific cell types will be used to unravel the contribution of these cells to the observed effects.

[O40] AhR Plasma Agonist Activity following Plant-Based versus Western Omnivorous Diets in Healthy Individuals

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Human plasma includes a variety of Aryl-Hydrocarbon-Receptor (AhR) ligands sourced from dietary intake and intestinal microbial activity. The presence and levels of these ligands can be influenced by dietary changes. Plant-based or vegan diets could positively influence immune responses and intestinal homeostasis in various inflammatory diseases, potentially by modulating the AhR activity. We hypothesized that a plant-based diet would alter gut microbiome composition and reduce AhR plasma agonist activity in contrast to effects of an unhealthy Western diet, as explored in our "Targeting AhR-dependent Inflammation for Organ Protection" consortium (TAhRget-Study, Identifier DRKS00031965). In addition, we hypothesized that the effects on AhR activity and microbiome composition promote a protective immune profile. We randomized 12 healthy female and male participants to follow contrasting dietary interventions for 3 days in a highly controlled inpatient environment at the DLR :envihab facility: either a whole food plant-based diet, or a Western diet (3 men and 3 women in each group). The plant-based diet was isocaloric, rich in anti- omega-3 fatty acids, contained low levels of saturated fats, cholesterol, and Omega-6 fatty acids, and a high fiber content. In contrast, the Western diet was hypercaloric, rich in salt, saturated fats, cholesterol, and pro-inflammatory n-6 fatty acids, with an unfavorable Omega-3 to Omega-6 ratio and a low fiber content. Throughout the study, we assessed physiological readouts and collected blood, urine, stool, and peripheral blood mononuclear cells (PBMCs) for deep phenotyping focusing on gut microbiome, immunity, and AhR activation markers. Using a cell based AhR luciferase reporter assay, we observed a longitudinal incremental reduction in AhR activity by 15±10% (repeated measurement two-way ANOVA time x diet, p=0.002) attributed to the whole food plant-based diet. These results were supported by a reduction in AhR target gene expression. Ongoing analyses include gut microbiome composition and function, immune phenotypes as well as AhR ligand profiles. Thereby we aim to identify dietary components that impact AhR-mediated inflammatory risk in health-to-disease transition.

[O41] Identification and characterisation of the importin complexes involved in the nuclear translocation of the aryl hydrocarbon receptor.

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The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor that mediates a broad spectrum of physiological processes in response to numerous substances including chemical pollutants, natural products and endogenous metabolites. Upon ligand binding, AHR undergoes translocation to the nucleus where it is directly implicated in the regulation of gene expression. The transport of the receptor into the nucleus relies on the specific interactions with the complexes of the importin proteins. So far, we are lacking a detailed description of the molecular mechanisms governing these interactions. In order to address this issue, we expressed and purified seven members of the human importin alpha family as well as the importin beta. Using immunoprecipitation technique, we identified the importin complexes involved in the interactions with AHR. We validated our findings using analytical size exclusion chromatography and nano-differential scanning fluorimetry. We also analysed the interactions in more details using fluorescence anisotropy and isothermal titration calorimetry. Structural characterisation of the complexes using X-ray crystallography and cryo-electron microscopy is currently under way. Our data provide first detailed insights into the molecular mechanisms governing nuclear translocation of AHR.

[O42] Identification of AHR activity-modulating drugs with potential involvement in cutaneous adverse drug reactions

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The occurrence of adverse drug reactions (ADR) is common and often poses a serious risk to the patient's health, as it regularly leads to discontinuation of the treatment. Additionally, there are often limited alternative treatments and management strategies available. Many adverse drug eruptions seem to be of an allergic nature, hypothesized to be initiated by drug-specific immune responses that activate T cells or induce IgE-mediated mast cell activation. However, most ADR do not require prior immune sensitization. Specifically, although immune cells play a role in off-target non-immune reactions, they are not driven by antigen-specific sensitization. Previously, our group demonstrated that vemurafenib antagonizes the aryl hydrocarbon receptor (AHR) signaling pathway, leading to the development of an off-target non-immune ADR characterized by inflammatory skin rashes. The interaction between a drug and the AHR is not exclusive to vemurafenib and may represent a more common and relevant mechanism of off-target non-immune ADR.

In the current study, our goal is to identify new AHR activity-modulating drugs with clinical relevance. Specifically, we concentrated on drugs previously associated with ADR like Stevens-Johnson syndrome. Furthermore, *in silico*-prediction models were used to identify drugs with potential AHR binding ability. Potential drugs were screened *in vitro* via reporter gene analyses which provided initial insights into whether the drug inhibits both basal as well as benzo[a]pyrene (BaP)-induced activity of the AHR-dependent luciferase construct. Furthermore, gene expression and enzyme activity of the AHR target gene CYP1A1 were determined after treatment with potential AHR-modulating drugs in HaCaT keratinocytes. First experiments with the kinase inhibitor capmatinib indicated an AHR-antagonistic effect. Capmatinib is used for treatment of metastatic non-small cell lung cancer and skin rashes are reported as common.

The data demonstrate that not only vemurafenib but also other drugs like capmatinib interact with the AHR *in vitro*. Since the AHR plays such a critical role in maintaining skin homeostasis and represents a key regulator in inflammatory processes, its role in cutaneous ADR needs further investigation. Furthermore, the understanding of underlying mechanisms and the identification of drugs inducing off-target non-immune ADR has immense clinical potential.

[O43] Jun dimerization protein 2 (Jdp2) is a spatiotemporal transcriptional activator of the AhR via the Nrf2 gene battery

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The crosstalk between aryl hydrocarbon receptor (AhR) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling is called the “AhR–Nrf2 gene battery”, works synergistically in reactive oxygen species (ROS) homeostasis to cell survival. Nrf2-dependent phase II enzyme gene promoters are controlled by coordinated recruitment of the AhR to adjacent dioxin responsive element (DRE) and Nrf2 recruitment to the antioxidative response element (ARE) within the promoter. The molecular interaction between AhR and Nrf2 members including phase I and II complexes, and their mediators are poorly understood. Here we demonstrated that AhR promoter was activated by phase I ligands included 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) through the AhR–Jdp2–Nrf2 axis in a time- and special transcription-dependent manner. Jdp2 was a bifunctional activator of phase I enzyme ligand- and phase II enzyme ligand-mediated transcription in response to TCDD. After TCDD exposure, Jdp2 activated the AhR promoter at the DRE and then moved to the ARE where it activated the promoter to increase reactive oxygen species (ROS)-mediated functions such as cell spreading and invasion in normal cells, and cancer regression in mutant kRAS–p53-driven pancreatic tumor cells. We conclude that Jdp2 plays a critical role in AhR promoter activation through the AhR–Jdp2–Nrf2 axis in a spatiotemporal manner. The AhR functions to maintain ROS balance and cell spreading, invasion, and cancer regression in a mouse model of mutant kRAS–p53 pancreatic cancer. These findings provide new insights into the roles of Jdp2 in the homeostatic regulation of oxidative stress and in the antioxidation response in detoxification, inflammation, and cancer progression.

(Refs) (1) Inflammatory and Regeneration 2023; 43: 42. (2) Cell Biol Toxicology 2022; 38(2): 203–222.

[O44] Straightforward Access to Novel Aryl Hydrocarbon Receptor Modulators through a Designed Multicomponent Reaction

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In this study, we developed novel indolocarbazole-based high-affinity ligands of the aryl hydrocarbon receptor (AHR) by employing Heterocyclic Multicomponent Reactions[1], as a highly efficient strategy to assemble tunable polyheterocyclic systems in a single step. Based on earlier findings of the group[2], a new multicomponent reaction was designed to readily synthesize a set of 6-substituted indolocarbazoles. In this approach, indole 2-carboxaldehyde and nucleophilic species directly yield the final adducts through a domino reaction. The scope of the process was analyzed and the range of the indole aldehydes and nucleophiles was established. 6-Formylindolo[3,2-*b*]carbazole (FICZ) analogues were readily prepared in one step out of commercially available precursors. Experimental and computational studies address the conformational behaviour of representative adducts, determining their potential chirality. Docking studies show the guidelines on the binding of model compounds with the AHR. Subsequent reporter gene analyses, gene expression analyses, and cytotoxicity assays revealed that these novel structures are potent activating ligands of the human AHR, importantly being non-toxic within the effective concentration range. Our approach enables the modulation of this target through a designed new chemistry and allow linking distinct moieties at solvent exposed areas. In this way, dimeric constructs were prepared, opening avenues for the generation of new derivatives at will.

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[O45] Endogenous aryl hydrocarbon receptor ligands regulate blood-brain barrier integrity

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Kynurenine pathway (KP) dysregulation is ascribed to the pathophysiology of various neuroinflammatory diseases. A hallmark of these diseases is blood-brain-barrier (BBB) dysfunction. However, our knowledge regarding the impact of KP metabolites on endothelial cells (ECs) of the BBB remains incomplete. In this study, we aimed at investigating the role of the KP on primary murine and human brain EC function. We report that inflammation induces expression of the KP enzymes indoleamine 2,3-dioxygenase-1 (IDO-1), kynurenine formamidase (KF) and kynurenine aminotransferases 1 and 3 (KAT1 and 3), resulting in accumulation of the KP metabolite kynurenic acid (KYNA). In experimental autoimmune encephalomyelitis (EAE), disease severity was ameliorated in EC-specific aryl hydrocarbon receptor (AhR) knockout mice. Here, we observed immune cell retention within paravascular cuffs likely restricting central nervous system (CNS) infiltration. Transcriptome analysis suggests altered phosphatidylinositol 3-kinase/protein kinase B (PI3K-Akt) signalling, and subsequent changes in focal adhesion (FA) and extracellular matrix (ECM) components, as underlying mechanism. Therefore, our findings argue for a potential novel role of the KP-AhR axis as regulator of BBB integrity.

[O46] Exploring the crosstalk between tryptophan metabolism and the aryl hydrocarbon receptor (AHR) in estrogen receptor positive breast cancer

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Breast cancer is the most frequent cancer in women worldwide. Eighty percent of patients suffer from estrogen receptor (ER)-positive tumors and are treated with endocrine therapies targeting the ER. Despite initial response to endocrine therapy, resistance evolves in a large proportion of patients. As the ER is a signaling molecule, research and clinical trials on therapy resistance so far mainly focus on the crosstalk of the ER with other oncogenic signaling networks. Consequently, compounds inhibiting signaling kinases are in clinical trials or clinical use for ER-positive breast cancer. However, the success of these interventions remains limited.

Only little is known about the contribution of tumor metabolism to endocrine therapy resistance. We are exploring the interplay of tryptophan metabolism with signaling networks to identify marker metabolites that predict relapse and guide targeted interventions. To this end, bioinformatics analyses of breast cancer gene expression data as well as analyses of mRNA and protein expression in breast cancer cells after treatment with ER activators or inhibitors were performed. Signaling pathways affecting tryptophan metabolism were modulated using pharmacological inhibitors or siRNA mediated gene knockdown and metabolite levels were measured. Our results reveal that modulation of tryptophan metabolism represents a novel undesired effect of endocrine therapy. Combining endocrine therapy with modulation of tryptophan metabolism or the AHR may prevent immune evasion and progression in ER-positive breast cancer.

[O47] Role of the AhR in Breast Cancer progression: a (micro)environment story…

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Breast cancer (BC) is a major public health concern, and its prognosis is very poor once metastasis occurs. The tumor microenvironment and chemical pollution have been suggested recently to contribute, independently, to the development of metastatic cells. The BC microenvironment consists, in part, of adipocytes and preadipocytes in which persistent organic pollutants (POPs) can be stored. We conducted an exploratory case-control study in which the concentrations of 49 persistent organic pollutants (POPs) were measured in both adipose tissue (AT) and serum samples from BC patients, with or without lymph node metastasis. The concentrations of several POPs in AT were positively associated with the risk of lymph node metastasis and the tumor size. We then developed a co-culture model using BC MCF-7 cells or MDA-MB-231 cells together with hMADS preadipocytes to investigate the contribution of the microenvironment and 2,3,7,8-tetrachlorodibenzo-p-dioxin TCDD, one of the identified POPs. Global differences were characterized using a high-throughput proteomic assay. Subsequently we measured the BC stem cell-like activity, analyzed the cell morphology, and used a zebrafish larvae model to study the metastatic potential of the BC cells. We found that coexposure to TCDD and preadipocytes modified BC cell properties; moreover, it induced the expression of ALDH1A3, a cancer stem cell marker, and the appearance of giant cancer cells with cell-in-cell structures (CICs), which are associated with malignant metastatic progression, that we demonstrated in vivo. The results of our study using BC cell lines co-cultured with preadipocytes and a POP and an in vivo zebrafish model of metastasis suggest that the interactions between BC cells and their microenvironment could affect their invasive or metastatic potential.

[O48] Investigating the role of the aryl hydrocarbon receptor on retinal function and morphology during aging and in age-related macular degeneration

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The Aryl hydrocarbon Receptor (AHR/Ahr) signaling pathway plays an important role in xenobiotic metabolism and detoxification throughout the body. Previously we demonstrated that the expression and activity of AHR in epithelial cells in the eye decreases with age. In the absence of the receptor, retinal function is compromised in aged mice who also present with key pathological features typical of age-related macular degeneration (AMD), a leading cause of vision loss in the elderly. RNA sequencing analyses of the ocular posterior pole revealed changes in the expression of genes associated with key AMD pathogenic pathways including inflammation, lipid metabolism, extracellular matrix regulation, and angiogenesis. The regulatory role of *Ahr* in ocular angiogenesis was examined in greater detail in full body *Ahr* KO mice following choroidal neovascular induction, which revealed a correlation between decreased Ahr activity and increased severity of neovascular lesions. Therapeutically, treatment with AHR agonists alleviated vascular leakage and slowed the development of neovascular lesions in aged wildtype mice subjected to laser induced neovascularization, a pre-clinical mouse model of the exudative sub-type of AMD. In the absence of *Ahr*, low-density lipoprotein levels were higher in the circulation (serum) and neutral lipids accumulated within the posterior pole of mouse eyes, supporting the role of *Ahr* in lipid regulation. Additionally, challenge with a high fat diet decreased the life span of *Ahr* KO mice. The *Ahr* signaling pathway was further investigated at the epithelial cell level using retinal pigment epithelial conditional KO mice (*Ahr* cKO). Mice tolerated a high fat diet challenge and presented with retinal and choroidal pathology including hypo- and hyper-pigmented regions in the posterior pole. The effect of *Ahr* cKO in epithelial cells was also examined in additional pathways associated with AMD development including inflammation, cell metabolism, lipid dysregulation and autophagy. Herein we have investigated the role of Ahr in combination with known AMD risk factors including advanced age and high fat diet and have identified a number of pathways regulated by this receptor in the eye and in AMD, providing further evidence in support of targeting it for potential therapy of retinal diseases in which the retinal pigment epithelial cells are compromised.

[O49] The tryptophan stress-induced mTORC1-AHR axis sustains autophagy and translation in tumors

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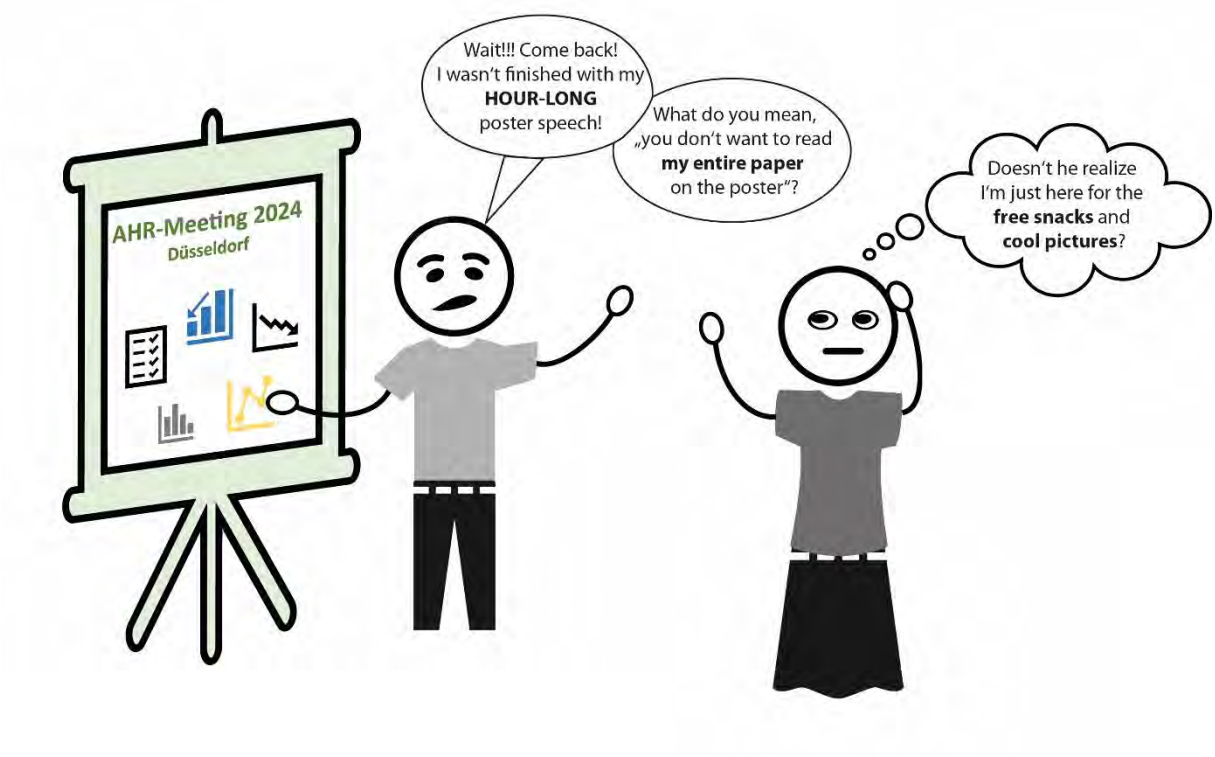
Trp is an essential amino acid that must be consumed with the diet and is important not only for AHR-related tumor-promoting functions, but also as a building block for protein synthesis and tumor growth. There is increasing evidence that Trp is heterogeneously distributed within the tumor and, as the least abundant amino acid, also rapidly decreases in concentration with distance from blood vessels. We found that AHR expression and activation are strongly induced upon Trp deprivation in human glioblastoma and breast cancer cells. Increased AHR expression is regulated by enhanced EGFR-RAS signaling, which activates the mTORC1-4E-BP1 and p38 MAPK pathways that promote the translation of the transcription factor ATF4. ATF4-driven transcription and 4E-BP1-sensitive translation synergize to induce expression of the AHR. The Trp-stress-induced mTORC1-AHR axis enhances macropinocytosis and autophagy, thereby sustaining intracellular Trp levels and the loading of tryptophanyl-tRNAs required for translation. The mTORC1-AHR pathway is also active in Trp-depleted areas of human tumors, and the Trp/ceramide emerges as a marker for response to drugs targeting this pathway.

NOTES

ABSTRACTS POSTER PRESENTATION



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[P1] Topical application of the cosmetic dye quinoline yellow affects AHR dependent gene expression in reconstructed human skin

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We recently discovered yellow quinophthalone dyes as arylhydrocarbon receptor (AHR) activators[1]. From the dyes investigated, quinoline yellow (QY), which is used in plastics, drugs, coloured smoke and cosmetics, was the most potent albeit transient AHR agonist when tested in breast cancer cell based assays.

Since its use is permitted for cosmetics according to the European cosmetics regulation dermal exposure to QY is likely. Other AHR ligands have been shown to induce the expression of genes of the epidermal differentiation complex and to accelerate keratinocyte terminal differentiation. In order to investigate possible effects of the quinoline dye in cosmetic products on the skin, we exposed commercially available 3D full-skin models to QY dissolved in liquid paraffin and examined the change in gene expression using microarrays. These models consist of dermis (fibroblasts), epidermis (keratinocytes) and stratum corneum (terminally differentiated keratinocytes). Topical exposure of the skin models to 0.01 % QY for 24 h caused an upregulation of the AHR-regulated genes CYP1A1 and CYP1B1, which suggests that QY can penetrate the stratum corneum barrier and activate AHR in keratinocytes. Among the 64 genes which were at least regulated two-fold, several genes such as SERPINB2, IL1B, IL24, TIPARP, HRNR, IL17RB and NQO1 have already been described to be AHR responsive. The upregulation of some of these genes was confirmed by qPCR and was dose dependent when assessed in additional experiments. In contrast, QY exposure had no effect on the expression of genes involved in cornification such as FLG, LOR or IVL. However, when the skin models were exposed for 7 days these genes were markedly upregulated as well. Consistent with these results the viable cell layer of the 3D-skin models decreased upon long term QY treatment as shown in histological analysis. In summary the data show that QY as AHR ligand affects skin differentiation in addition to inflammatory responses. Future research is necessary to investigate similarities or differences of various AHR ligands the skin may be exposed to.

[1]Tarnow et al. Chem Res Toxicol 33(3):742-750

[P2] The AHR/CYP1A1 Axis is Dysregulated at Multiple Levels in Psoriasis

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The Aryl Hydrocarbon Receptor is a ligand-activated transcription factor and environmental sensor with homeostatic and anti-inflammatory roles in skin. Upon activation, AHR induces the expression of the cytochrome P450 (CYP1) enzyme family, which provides critical negative feedback by degrading AHR ligands. We have previously shown that excessive CYP1A1 activity impairs AHR's anti-inflammatory role in psoriasis-like skin inflammation in mice, while suppression of aberrant enzymatic activity restores AHR's beneficial effect. Also, CYP1A1 activity is significantly enhanced in Th17 cells of psoriasis patients, suggesting that dysregulation of CYP1A1 activity may play a role in psoriasis.

Here, we performed a multi-level investigation of the AHR/CYP1A1 axis in the blood and skin of psoriasis patients and healthy volunteers, assessing AHR ligand and precursor availability by mass spectrometry, *AHR* and *CYP1A1* mRNA expression in blood and skin by qRT-PCR and spatial expression in skin by RNAscope, *AHR* mRNA expression in normal human epidermal keratinocytes (NHEK) following TNF (10ng/mL) and IFN-γ (10ng/mL) stimulation by qRT-PCR, and CYP1 enzymatic activity by the EROD assay.

Serum levels of the ligand precursor tryptophan were significantly decreased in psoriasis patients ($P<0.001$; n=20) versus healthy (n=20). *AHR* mRNA expression was significantly decreased in both psoriasis whole blood ($P<0.01$; n=18) and psoriasis lesional (PsL; $P<0.01$; n=18) skin, while *CYP1A1* mRNA expression did not differ in whole blood but showed a downward trend in PsL ($P=0.0546$; n=18). *AHR* expression predominantly localised in the epidermal basal layer in PsL (n=6) compared to a more widespread expression in healthy skin (n=8), with a reduction in *AHR* positive cells in the PsL dermis ($P<0.05$). TNF ($P<0.001$) and IFN-γ ($P<0.0001$) significantly upregulated *AHR* mRNA (n=4 wells) expression in NHEK. *In vitro* culture of skin epidermal sheets with the AHR agonist FICZ (5nM) significantly upregulated *CYP1A1* mRNA expression in healthy ($q<0.05$; n=8) and psoriasis epidermis ($q<0.05$; n=8), however CYP1 enzymatic activity was not increased by AHR ligation in PsL. In contrast, CYP1 enzymatic activity was enhanced in Th17 cells from the same patients ($P=0.0535$; n=8).

Taken together, our data show that there is a multi-level dysregulation of the AHR/CYP1A1 axis in psoriasis and suggests a failure of multiple pathway checkpoints that could be targeted to restore AHR's beneficial effects in skin.

[P3] The skin commensal yeast *Malassezia* modulates tissue homeostasis via the aryl hydrocarbon receptor

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With its large surface the skin protects the body from dehydration, entry of harmful substances and infection. Commensal microbes colonizing the skin contribute to tissue homeostasis by shaping protective immunity and strengthen the barrier function of the skin. Surprisingly, the fungal community of the skin microbiome is dominated by a single fungal genus: the lipophilic yeast *Malassezia*. Our understanding of the interaction between *Malassezia* and the host remains incomplete, both in normal and inflamed skin conditions. Interestingly, *M. furfur* converts tryptophan into indoles that can act as ligands for the aryl hydrocarbon receptor (AhR). Understanding the consequences of *Malassezia*-induced AhR signaling is of relevance in light of the association of the fungus with diverse skin disorders characterized by barrier impairment and inflammation, such as atopic dermatitis and seborrheic dermatitis. By combining *in vitro*, *ex vivo*, and *in vivo* approaches we discovered that tryptophan-induced fungal metabolites exert a host protective role in the colonized skin. More specifically, we found that *Malassezia*-derived indoles modulate via AhR the expression of structural proteins in human epidermal equivalents and murine epidermal sheets. In an experimental skin colonization model in mice, exposure of barrier-disrupted skin to *Malassezia* limited inflammation and promoted barrier repair in an AhR dependent manner. Together, our results suggest that that AhR acts as a molecular sensor for commensal fungi that contributes to the restoration of homeostasis in the inflamed skin.

[P4] Disruption of AhR signaling alters differentiation and production of surfactant in human alveolar epithelial cells

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AhR plays a central role responses of lung cells towards toxic insults of airborne pollutants, such as polycyclic aromatic hydrocarbons (PAHs) in the respiratory tract. Surfactant lipids and proteins, which cover alveolar epithelium, both reduce surface tension and provide protection to pneumocytes. Here, we studied potential role of AhR in the production of surfactant in a human model of ATII cells, A549 cell line, and their differentiation towards more advanced ATII-like phenotype, when cultivated at air-liquid interface (ALI). Using AhR knock-out (AhR KO) cells derived from A549 cells and wild-type cells, we found that AhR KO cells expressed lower levels of principal surfactant protein SP-C in its various forms, had lower levels of phospholipids, including several phosphatidylcholine species, which was accompanied by a reduced expression of fatty acid synthesis and phospholipid synthesis proteins, such as FASN, SCD, CCTα and LPCAT1. Moreover, the lack of the AhR limited the ability of lung epithelial cells to differentiate at ALI cultivation conditions, which had lower numbers and less mature lamellar bodies, as well as decreased levels of lamellar bodies markers (ABCA3, LAMP3). These effects might be linked with increased levels of a principal regulator of lung growth - transcription factor SOX9 in AhR KO cells, and with enhanced autophagy associated with pro-proliferative phenotype in lung epithelial cells. Finally, we observed that in human embryonic stem cells-derived expandable lung epithelium cells, lack of AhR was also associated with decreased SP-C and ABCA3 expression. These results suggest that airborne toxicants, such as PAHs might contribute to disruption of ATII cell functions via AhR activation and alteration of proliferation – differentiation equilibrium and surfactant production.

Funded by the Czech Science Foundation, project no. 18-00145S.

[P5] Electrical impedance spectroscopy evaluates organotypic epidermis formation, response to AHR activation, and skin barrier function *in vitro*

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Investigative dermatology benefits from human epidermal equivalent (HEE) cultures as advanced *in vitro* models. Non-intrusive quantification of skin barrier development and functionality would increase study reproducibility and allow to assess HEE development before commencing experiments. Here, we investigated the utility of an electrical impedance spectroscopy (EIS) device equipped with a customized smart-lid fitting a 24-transwell cell culture system. Measuring impedance across 10Hz to 100kHz, we identified two frequency ranges of interest in the resulting impedance spectra: a lower frequency range correlated strongly with keratinocyte terminal differentiation (termed EIS^{diff}) and a higher frequency range correlating with *stratum corneum* thickness (termed EIS^{SC}). In N/TERT-2G immortalized keratinocytes, CRISPR/Cas9-based knockout of terminal differentiation proteins filaggrin and claudin-1 reduced epidermal barrier formation and impedance values (44% and 73% EIS^{diff} reduction, respectively). HEEs treated with pro-inflammatory cytokines IL-4 and IL-13, simulating atopic dermatitis *in vitro*, reduced EIS^{diff} and EIS^{SC} by 30% and 16%, respectively. AHR-activating ligands (coal tar, SGA388, IMA-7101, and TCDD) reversed the EIS^{diff} reduction completely, while AHR-binding non-activators (SGA360 and teriflunomide) further decreased EIS^{diff} values (12% and 18% additional EIS^{diff} reduction, respectively), likely by blockage of endogenous AHR signaling. As expected, CRISPR/Cas9-based knockout of AHR and TFAP2α impairs keratinocyte terminal differentiation and markedly reduced impedance values (55% and 59% EIS^{diff}, and 34% and 46% EIS^{SC} reduction, respectively). EIS offers a standardized, high-throughput method for non-invasively monitoring of HEE barrier formation and function. It effectively quantifies the impact of AHR signaling on keratinocyte terminal differentiation and skin barrier development under healthy and inflammatory disease conditions. Future use of impedance spectroscopy, for example to quantify the effects of the microbiome on HEE development and barrier function, is currently under investigation.

[P6] Regulatory Mechanism of the IL-33-IL-37 Axis via Aryl Hydrocarbon Receptor in Atopic Dermatitis and Psoriasis

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Interleukin (IL)-37 suppresses systemic and local inflammation. It is expressed in the epidermis, the external layer of the skin, and is decreased in inflammatory skin diseases including atopic dermatitis (AD) and psoriasis. Therefore, an agent applied topically on the skin that can increase IL-37 could be promising for treating AD and psoriasis; however, the mechanism regulating IL-37 remains largely unknown. Given that IL-37 expression is induced in differentiated keratinocytes, a major component of the epidermis, and that activation of aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor, promotes keratinocyte differentiation, we hypothesized that AHR might be involved in the IL-37 expression in human keratinocytes. We analyzed normal epidermal human keratinocytes (NHEKs) treated with tapinarof, a potent AHR modulator. We found that tapinarof treatment upregulated IL-37 in NHEKs, which was canceled by the knockdown of AHR using siRNA transfection, indicating that AHR mediates IL-37 expression in NHEKs. Furthermore, we found that the knockdown of IL-37 resulted in the upregulation of IL-33, an alarmin cytokine with crucial roles in the pathogenesis of AD and psoriasis. These findings suggest that IL-37 negatively regulates IL-33 expression in NHEKs. Finally, we examined whether tapinarof treatment modulates IL-33 expression in NHEKs. Tapinarof treatment inhibited IL-33 expression, which was partially reversed by the knockdown of either AHR or IL-37. Also, It has been reported i) that IL-33 expression is partially dependent on mitogen-activated protein kinase (MAPK) activation, and IL-37 has a role in suppressing MAPK, and ii) that IL-33 downregulates skin barrier function proteins including filaggrin and loricrin, thereby downregulating the expression of IL-37, which colocalizes with these proteins. This leads to an imbalance of the IL-33-IL-37 axis, involving increased IL-33 and decreased IL-37, which may be associated with the pathogenesis of AD and psoriasis. Therefore, AHR-mediated regulation of the IL-33-IL-37 axis may lead to new therapeutic strategies for the treatment of AD and psoriasis.

[P7] Regulation of the AhR signaling pathway during skin allergy

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The aryl hydrocarbon receptor (AhR) has a well-known role in regulating skin barrier function and allergic reactions. Its activity is regulated by the AhR repressor (AhRR) and cytochrome P450 (CYP) enzymes that metabolize AhR ligands. We have recently shown that the AhRR is highly expressed in immune but not epidermal cells suggesting a different regulatory mechanism for AhR in skin cells. Therefore, the molecular mechanisms and therefore beneficial or adverse consequences of dermal AhR activation are not entirely understood. Here we show how depletion of AhR regulator AhRR onset and pathology of allergy models, such as contact hypersensitivity (CHS) and atopic dermatitis (AD). Mice lacking AhRR display an altered adaptive and innate immune response that affects disease progression. Thus, our findings will help to understand the mechanism of regulation of AhR activation by AhRR and CYP enzymes in the skin allowing future development of therapeutic agents for AD that target the AhR signaling pathway in the skin.

[P8] Evidence for a role of the aryl hydrocarbon receptor identified in a simple 2-D model of systemic sclerosis

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New therapeutic approaches are urgently needed for systemic sclerosis (SSc), a rare, challenging and currently incurable autoimmune disease characterized by hardening of the skin and internal organs, usually due to fibroblast changes. Aryl hydrocarbon receptor (AHR) is critical for skin homeostasis, but very little is known regarding SSc. TGFß, a known target gene of AHR, is also implicated in SSc. Here, we investigated the impact of AHR deficiency on the production of matrix metalloproteinases 1 protein (MMP1) and collagen type 1 (COL1A1) in TGFß-treated dermal fibroblasts.

Wild-type human dermal fibroblast (wt HDF) and keratinocyte (wt HaCat) cell lines, along with AHR-negative human fibroblast (AHR-KO HDF) and keratinocyte (AHR-KO HaCat) cell lines, were used in the study. AHR-KO HDF and AHR-KO HaCat cell lines were generated with Crisp/Cas9. Cells were treated with TGFβ (10 and 25ng/ml) for 24h/48h. Differential gene expression was determined using Droplet Digital PCR (ddPCR™). COL-1A1 and MMP1, two fibrosis-relevant proteins, were measured by ELISA. Expression of AHR and β-catenin was assessed by western blotting. Co-cultures of AHR-KO/wt HDF and AHR-KO/wt HaCaT were studied to answer the question how activation or lack the AHR affects the HDF capacity to secrete MMP1 or collagen proteins.

As expected, wt HDF produced at basal level more COL1A1 compared to HaCaT cells, and TGFß induced more COL1A1 secretion in HDF cells. Lower levels of COL1A1 were detected in AHR-KO HDF compared to wt HDF. This trend was evident both at the baseline (comparing the control groups) and after treatment with TGFβ. Moreover, FICZ reduced secretion of COL1A1 in wt HDF. AHR protein itself also increased upon TGFß treatment. Curiously, adding the high-affinity AHR-agonist, FICZ to the wt HDF reduced AHR protein, but not AHR mRNA. Finally, both at baseline and following TGß treatment, the secretion of MMP1 in AHR-KO HDF cells was lower compared to wt HDF. Additionally, the lack of AHR in co-culture had a significant impact on decreased MMP1 secretion.

In conclusion, COL1A1 secretion increased in a simple SSc model based on treatment of HDF with high concentrations of TGFß. Both deficiency of AHR and activation of the AHR signaling pathway by FICZ affected expression levels of MMP1 and COL1A1 induced by TGFß. As AHR expression itself was also sensitive to TGFß, this may reflect on a role exploitable in the regulation of the fibrosis process, which needs to be studied further.

[P9] Tryptophol (IEt) from Skin Commensal *Cutibacterium Acnes* Promotes Epidermal Proliferation and Differentiation by activating AHR-ALDH1A3 Pathway

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The Aryl Hydrocarbon Receptor (AHR) is a key transcription factor that reacts to environmental stimuli and plays a crucial role in maintaining epithelial homeostasis. Recent research has shed light on its novel function in the interaction between microbes and their hosts. Skin commensals, or their metabolites derived from tryptophan, can influence the functions of the skin barrier through the AHR signaling pathway. However, the exact microbial species involved and their specific molecular mechanisms have not been fully explored.

In this study, we discovered that ALDH1A3, an enzyme vital for retinoic acid metabolism, is a target gene of AHR in Keratinocytes. We observed that when AHR-positive HaCaT cells were treated with Benzo(a)pyrene (BaP), a well-known AHR agonist, there was an increase in ALDH1A3 expression and retinoic acid levels. This response was absent in AHR-negative HaCaT cells. Additionally, this phenotype was also confirmed in vivo. Applying BaP on mouse skin resulted in increased skin thickness, which was attributed to enhanced skin cell proliferation and differentiation.

Subsequently, our research showed that the AHR-ALDH1A3 pathway is not exclusively triggered by BaP. When cells were exposed to filtrates from three prominent skin commensals in the Han Chinese population, only *Cutibacterium acnes* was found to specifically activate the AHR/ALDH1A3 pathway. Moreover, using liquid chromatography-mass spectrometry (LC-MS), we identified IEt (Tryptophol), a unique tryptophan metabolite produced by *C. acnes*, which could activate AHR and increase ALDH1A3 expression, thus altering skin retinoic acid metabolism.
In summary, the tryptophan derivative IEt from *C. acnes* triggers the AHR/ALDH1A3 pathway, impacting the host's metabolism of retinoic acid. These findings emphasize the significant role of AHR in the microbial regulation of host metabolism and suggest that the AHR axis is a promising area for further research into microbial-host interactions.

[P10] Tracing gut microbial tryptophan metabolism by ^{19}F NMR

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The gut microbiota plays an essential role in maintaining host immune homeostasis. Many of these effects are mediated by the binding of tryptophan and its metabolites to the aryl-hydrocarbon receptor (AhR). Tryptophan is an essential amino acid which may be metabolized through different pathways to at least 30 different small molecules. Profiling of these metabolites can provide a better understanding of biological systems and disease mechanisms. Even though ^{1}H NMR has been extensively used in clinical research for metabolic profiling, this method suffers from high background and narrow chemical shift range, which makes the identification of metabolites difficult. This limitation may be overcome by labelling of tryptophan with a stable NMR isotope such as ^{19}F that shows low natural abundance, high sensitivity and a large chemical shift range (1). Thereby, signals from molecules not related to tryptophan metabolites are eliminated and signal identification is greatly facilitated.

Here, we used ^{19}F tryptophan tracing utilizing 5-fluoro-tryptophan for studying the metabolism of tryptophan by different human gut microbial species such as *Bifidobacterium longum* subsp. *longum, Bacteroides thetaiotaomicron,* and *Bacteroides fragilis* in a time dependent and quantitative manner. Furthermore, cecum content of both germ-free and specific pathogen-free (SPF) mice fed with ^{19}F labeled tryptophan will be analyzed. One major challenge is the assignment of resulting NMR signals to specific metabolites. To this end, we combine data from high-resolution mass spectrometry with measurements of standards and chemical shift predictions to derive a catalogue of tryptophan metabolites with their corresponding ^{19}F spectral positions.

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[P11] Involvement of the aryl hydrocarbon receptor in action mechanism of fungal secondary metabolites with antiviral activity against animal coronaviruses

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The aryl hydrocarbon receptor (AhR) influences immune response to various viral infections, affecting host resistance and survival. It is involved in the host response to coronaviruses (CoVs), such as murine coronavirus (MCoV), Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome (SARS-CoV-1), severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), human coronavirus (HCoV) 229E, and canine coronavirus (CCoV), and AhR antagonists counteract CoV infection in several mammalian host cells. Interestingly, our previous studies have demonstrated that 3-*O*-methylfunicone, penisimplicissin and vermistatin, which are funicone-like compounds produced by the fungus *Talaromyces pinophilus*, have antiviral activity against CCoV, potentially inhibiting AhR.
The results of the present study showed that AhR, expressed in feline (CRFK) cells as well as in bovine (MDBK) cells, is considerably activated by feline (FCoV) and bovine (BCoV) coronaviruses infections, and that the selective AhR antagonist CH223191 reduces *in vitro* replication of these CoVs, responsible for both enteric and respiratory diseases in cats and cattle, respectively.
Moreover, in order to describe the molecular determinates driving the AhR recognition mechanism by different funicones during CoVs infection, we applied a combined approach in which Molecular Docking calculations have been integrated with homology modelling methodologies. Taking advantage of our computational strategy, we explored the molecular forces involved in the stabilization of the complex of AhR with the different funicones.
These findings represent a fundamental step for the development of innovative drugs having AhR as a potential target for therapy against CoVs.

[P12] Asthma's Hidden Mechanism: AhR Regulation of Mucus Production and Inflammasome

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While the significance of mucus overproduction in asthma has long been recognized, its precise cause remains poorly understood. Muc5ac is a secretory mucin known to be associated with reduced pulmonary function and asthma exacerbations. The research sought to unravel the immunological mechanisms governing Muc5ac expression and allergic airway inflammation in asthma. We observed that exposure to cockroach allergens led to an increased expression of Muc5ac in human bronchial epithelial cells (HBECs) and in a mouse model of asthma. Furthermore, the study identified heightened levels of AhR (Aryl Hydrocarbon Receptor) and its downstream genes CYP1A1 and CYP1B1 in response to cockroach allergen exposure. Notably, mice lacking AhR exhibited exacerbated allergic airway inflammation and increased MUC5AC expression. Additionally, cockroach allergen exposure triggered the activation of the NLRP3 inflammasome in the airways, as evidenced by the upregulation of NLRP3, Caspase-1, and IL-1β, indicating an inflammatory response. Interestingly, this inflammasome activation was intensified by AhR knockdown or the AhR antagonist CH223191. Moreover, the study found that the absence of AhR in HBECs resulted in elevated reactive oxygen species (ROS) production, particularly Mito-ROS, which, in turn, appeared to drive inflammasome activation. Importantly, the inhibition of ROS or Mito-ROS effectively suppressed NLRP3 inflammasome activation. Remarkably, inhibiting the NLRP3 inflammasome with MCC950, a specific inhibitor, showed promise in mitigating allergic airway inflammation and reducing Muc5ac expression. Additionally, the research unveiled IL-1β as a key mediator in the induction of Muc5ac expression by cockroach allergens in HBECs. Collectively, these results reveal a previously unidentified functional axis of AhR-ROS-NLRP3 inflammasome in regulating Muc5ac expression and airway inflammation.

[P13] Influence of the AhR/AhRR Pathway and Environmental Chemicals on Macrophage Programming

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The aryl hydrocarbon receptor (**AhR**) is a ligand-activated transcription factor with important roles in toxicology and cell physiology. The AhR is a known mediator of the toxic effects of dioxins, but multiple ligands are also provided by the diet. Importantly, AhR signaling has to be strictly regulated, amongst other mechanisms, through a negative feedback loop involving the transcription of the AhR repressor (**AhRR**).

We previously discovered that the AhRR mainly regulates AhR activity in immune cells rather than non-hematopoietic cells. Here, we characterize the effects of AhR activation through several ligands (3MC, FICZ, ICZ, and BpA) in WT and AhRR$^{-/-}$ bone marrow-derived macrophages (BMM) and determine the kinetics of AhR signaling activity. Investigating the role of AhRR in macrophages more closely, Fetal Liver Macrophages (FLiM) were cultured and examined by bulk-RNA Sequencing and Seahorse metabolic analysis. AhRR-deficient FLiM cells showed an enrichment in mTORC1 and Myc pathways, known as master regulators of protein synthesis and cellular metabolism. Mitochondrial stress test analysis confirmed metabolic changes in AhRR-deficient FLiM cells and BMM towards a phenotype preferring oxidative phosphorylation. Furthermore, BMM revealed an increase in ROS production and altered lipid composition with a major difference in TAG content in the absence of AhRR. Overall, these data show that AhRR-deficient macrophages exhibit changes in their immunometabolic phenotype with increased mitochondrial capacity, suggesting AhRR as a rheostat for cellular metabolism.

To prove this *in vivo*, we analyzed the role of AhRR in whole-body energy metabolism using a diet-induced obesity model. In line with our *in vitro* results, we show that AhRR-deficient mice are protected from developing severe metabolic syndrome, accompanied by increased energy expenditure and ameliorated hepatosteatosis. Therefore, the AhR/ AhRR signaling axis regulates cellular and whole-body energy metabolism and could be a potential target to address the increasing prevalence of metabolic diseases.

[P14] AhR agonism by tapinarof regulates T_H2 and T_H17 cell function in human skin

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The aryl hydrocarbon receptor (AhR) is a transcription factor for skin homeostasis and barrier function. Tapinarof, a topical AhR agonist, has shown impressive clinical efficacy in psoriasis (PSO) and atopic dermatitis (AD), inducing long-lasting remissions. However, tapinarof's anti-inflammatory mechanism remains unclear. We aimed to investigate tapinarof's effects on T cells in healthy skin, AD, PSO, and allergic contact dermatitis (ACD).

Using a short-term human skin explant model, we cultured skin biopsies from PSO, AD and ACD with tapinarof for 24 hours. We observed elevated cytokine levels in disease-driving populations of tissue-resident T cells (T_{RM}) (IL-13$^+$CD4$^+$ T_{RM} in AD and IL-17a$^+$CD8$^+$ T_{RM} in PSO), validating our model. Tapinarof significantly reduced IL-13 and IL-17a in the respective diseases and populations. In ACD, tapinarof decreased IL-13 levels in T_{RM} and CD4$^+$ T cells without affecting IFN-γ expression.

By single-cell RNA-sequencing of T cells isolated from tapinarof-treated AD and PSO biopsies, we found that these cells displayed significant metabolic impairments. These findings were corroborated by transcriptomic analysis of tapinarof-treated PBMCs and CD4$^+$ memory T cells, which interestingly showed a strong concerted downregulation of fatty-acid beta-oxidation and cholesterol metabolism.

Mechanistic studies confirmed that glycolysis and oxidative phosphorylation were reduced in resting and activated memory T cells after tapinarof treatment. Strikingly, basal respiration was significantly impaired in both resting and activated memory T cells, whereas basal glycolysis was only affected after activation. Since memory T cells rely heavily on oxidative phosphorylation for energy production, treating memory T cells with tapinarof could lead to a metabolic impairment.

In summary, our ex vivo model demonstrates that treatment with tapinarof significantly reduces disease-relevant cytokines in skin T cells from AD,PSO and ACD biopsies. Furthermore, we provide mechanistic evidence that this is mediated by impairment of glycolysis and oxidative phosphorylation, revealing a previously unknown mechanism of action.

[P15] Investigating microbiome-AhR interactions underlying CD4$^+$ T cell differentiation

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One link between the environment and autoimmune disease is the aryl hydrocarbon receptor (AhR), a ligand activated transcription factor that can bind a diverse repertoire of ligands including environmental chemicals, diet-derived ligands, and microbiota metabolites. In a variety of autoimmune disease mouse models, AhR activation has been linked to the induction of both proinflammatory T helper 17 cells (Th17) cells and regulatory T cells (Tregs). However, the factors leading to Th17 or Treg differentiation are not fully understood. Using the non-obese diabetic (NOD) model for type 1 diabetes (T1D), studies in our laboratory have shown that AhR activation by indole-3-carbinol, a diet-derived ligand, leads to an increase in proinflammatory Th17 cells, insulitis, and changes in the gut microbiome. In contrast, AhR activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin, an environmental chemical, increases immunosuppressive Tregs, decreases Th17 cells and prevents T1D. Since these ligands can activate AhR in the intestine where it can impact mucosal immune cell development, cell differentiation, production of antimicrobial compounds, and microbiome composition, the overarching hypothesis is that the outcome of CD4$^+$ T cell differentiation following exposure to AhR ligands is driven by interactions with the gut microbiome. To test this hypothesis, we analyzed CD4$^+$ T cell differentiation in three different NOD mouse models: 1) AhR knockout (KO) mice, 2) gnotobiotic mice, and 3) transgenic mice with islet antigen-specific CD4$^+$ T cells. We observed AhR KO mice had a delayed onset of in hyperglycemia, an increase in Tregs when compared to AhR wildtype (WT) NOD mice under specific pathogen free conditions, and microbial changes in the gut. However, under germ free conditions there were no differences in Tregs and development of hyperglycemia. In the transgenic model, we found that diabetogenic T cells were found in the small intestine and pancreatic lymph nodes, and modulated by AhR activation, suggesting AhR ligands directly modify antigen specific CD4$^+$ T cells. Ultimately, the data will help elucidate how activation of AhR influences CD4$^+$ T cell differentiation, identify how environmental factors contribute to T1D susceptibility, and uncover how AhR can be targeted for T1D prevention.

[P16] The contribution of diet and microbiome to AhR modulation along the murine gastrointestinal tract

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The AhR is a ligand-dependent transcription factor located at physiological barriers and expressed in immune cells. It responds to the environment, generates a variety of pathological and beneficial downstream effects and is involved in inflammatory disorders. AhR signaling is influenced by endogenous ligands, as well as ligands of dietary or microbial origin. In this study we aimed to characterize the contribution of the diet and the microbiome to the modulation of the AhR in the intestine. To assess the influence of the microbiome, we compared colonized pathogen-free (SPF) or germ-free (GF) mice (C57BL/6J). Using a cell based AhR luciferase reporter assay we show that AhR activation is significantly higher in the serum of SPF compared to GF, highlighting the importance of microbiome-derived ligands. Microbiome-dependent differences were also investigated segment-specifically by using luminal contents and intestinal tissue of SPF and GF. We observed that luminal contents from all intestinal segments of SPF mice activate the AhR in vitro, with AhR activation markedly increasing in the caecum and colon. By contrast, contents from GF mice displayed an overall lower AhR activation, with highest values in the stomach while being drastically lower in the caecum and colon. Intestinal *Cyp1a1* expression confirmed this pattern on intestinal segment level.
Our data highlight the importance of the microbiome and dietary components for the modulation of AhR-dependent processes in the mouse gut.

[P17] Homeostatic activation of Aryl Hydrocarbon Receptor in monocytes controls systemic tonic type I interferon responses

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The ligand-activated transcription factor Aryl Hydrocarbon Receptor (AhR) exerts pleiotropic effects in multiple immune cells. However, AhR function in monocytes remains poorly understood. Here, we show that AhR regulates spontaneous interferon beta (IFN-β) production in both human and mouse monocytes. In vivo in mouse, AhR deletion in monocytes unleashed systemic type I interferon responses in the steady-state. This effect was decreased by antibiotics treatment, indicating that basal IFN-β production in monocytes is sustained by microbiota-derived products and that homeostatic AhR signalling represses this pathway, thereby restraining tonic type I interferon responses. Moreover, this phenomenon was specific to monocytes, as mice deficient for AhR specifically in macrophages did not show any dysregulation of interferon-stimulated genes. In vivo in human, low AhR activity correlated with elevated type I interferon responses in monocytes from patients with systemic juvenile idiopathic arthritis. Microbiota-induced IFN-β production by monocytes has been shown to be essential for the efficacy of anti-cancer therapies, including checkpoint blockade. In mice deficient for AhR in monocytes, we demonstrated increased efficacy of anti-PD1 therapy in a pre-clinical fibrosarcoma model, showing that monocytes could be a target of AhR inhibition for anti-tumoral therapies. In conclusion, our findings show that homeostatic activation of AhR in monocytes restrains the spontaneous production of IFN- β, and is essential for fine-tuning tonic type I responses. Our work sheds new light on the role of AhR in monocytes and refines the rationale for manipulating its activity for disease treatment.

[P18] Role of the aryl hydrocarbon receptor in immunosurveillant functions of murine dendritic epidermal T cells

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Mouse epidermis harbors about 2-5% of resident Vγ5Vδ1 T cells, called dendritic epidermal T cells (DETC) due to their morphology. These thymus-derived cells seed the skin around birth and are involved in skin homeostasis, immune surveillance and wound healing. DETC are not MHC-restricted and quickly respond to activation with secretion of cytokine, chemokines or growth factors, thereby recruiting inflammatory cells into the skin or communicating with keratinocytes and/or Langerhans cells. We previously showed in aryl hydrocarbon receptor (AHR)-deficient mouse pups that AHR is necessary for expansion of incoming DETC in the neonate skin. Moreover, AHR-absence increased gene expression of inflammatory pathways in DETC sorted from very young AHR-deficient mice. To address the question, whether AHR dampens the inflammatory potential in DETC from adult mice as well, we used a novel tamoxifen-inducible DETC-specific AHR deletion mouse model. Adult 8- to 10-week-old mice were injected i.p. with tamoxifen, which eliminated the AHR in up to 90% of the DETC within three weeks. The mice were then tested for possible barrier defects. Transepidermal water loss (TEWL) was measured to test barrier integrity. We found that lack of AHR in DETC did not impair this parameter. Possibly, the moderated tape-stripping of the skin, used to initiate TEWL, is too minor to involve DETC in repair exerted by the keratinocytes. To gain data on AHR-involvement in DETC functions, we performed RNAseq from DETC isolated after induced AHR-deletion. In contrast to our expectation and the data of the DETC from pre-puberty mice, inflammation-related genes were not central to gene changes upon AHR-deletion in the adult mice. However, we identified many downregulated genes associated with cell division, such as: Ticrr, Ckap2l, Sapcd2 and Wdr35. This is consistent with in vitro studies with the DETC cell line 7-17 and its AHR-deficient derivative. Here, an altered proliferation rate was observed. Furthermore, we found evidence for a decrease in total ATP production upon AHR deletion and a depletion of mitochondria, which could be correlated with increased ROS-production, and have consequences for the energy-demanding proliferation capacity. Although the study is ongoing, the current data strongly suggest that AHR is a critical molecule also in adult DETC function, which warrants further investigation.

[P19] High-dimensional investigation on the impact of oral *Indigo naturalis* on aryl hydrocarbon receptor-regulated immune responses in healthy volunteers

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Indigo naturalis (IN), a herbal medicine containing indole alkaloid AHR ligands, is a promising oral therapeutic for patients with ulcerative colitis (UC). Its observed effects in treating UC have been linked to AHR activation in tissues of the intestinal microenvironment. Despite being a promising AHR-modulating therapeutic for UC, its impact on other targets outside of the gastrointestinal tract remains underexplored and deserves investigation, particularly as the unsupervised use of IN is associated with systemic adverse events in humans, including pulmonary hypertension, recently linked to AHR activation[1]. We report a dose-escalation clinical study (NZ-INDES, ACTRN12621000097842) involving 25 healthy volunteers allocated to receive a single capsule containing either placebo (2 g microcrystalline cellulose), 0.25 g, 0.5 g, 1 g or 2 g IN. Blood samples were sampled at 0, 1, 3, 6 and 24 h following placebo or IN ingestion. To determine the relationship between IN-induced AHR-regulated molecular responses, cellular immune responses and bioavailable IN-derived metabolites, we comprehensively analysed sampled blood plasma and peripheral blood mononuclear cells (PBMC). The methodology included single-cell RNA sequencing and high-dimensional flow cytometry analyses of PBMC; coupled with targeted mass-spectrometry-based plasma metabolomics and assessment of net AHR agonistic activity in plasma by means of a cell-based reporter assay. We aim to present a detailed summary of our work and present findings of potential relevance for the pathophysiology of pulmonary hypertension observed following acute IN intake in healthy volunteers.

[1]Masaki, T., *et al.* (2021), *PNAS, 118*(11), e2023899118

[P20] Unravelling the role of AhR-associated inflammation in experimental chronic kidney disease

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Cardiovascular disease (CVD) is the leading cause of mortality in patients with chronic kidney disease (CKD) - an association that receives insufficient attention to date and has not yet been translated into targeted therapies. Given the substantial prevalence of CKD, novel therapies are needed. Chronic pro-inflammatory mechanisms are known to be main contributors, the triggers of which are insufficiently described. We hypothesize that the microbiome and its metabolites are drivers of inflammation and cardiovascular risk in CKD, and a promising therapeutic target. Recently, we showed that patients with CKD show signs of inflammation alongside gut barrier dysfunction associated with gut dysbiosis and increased plasma levels of tryptophan (Trp) metabolite indoxyl sulfate (IxS). Trp metabolites, known to act as aryl hydrocarbon receptor (AhR) ligands and recognized for their immunomodulatory properties, accumulate in CKD. Thus, this study aims to investigate the role of AhR-associated inflammation in CKD.

In a murine model, we induced CKD by surgically reducing renal mass by 5/6 nephrectomy. CKD mice exhibited pronounced cardiovascular impairment, concomitant with increased serum levels of uremic toxins, particularly indoxyl sulfate (IxS), and an associated pro-inflammatory immune response. Interestingly, depletion of the microbiome through administration of a non-absorptive antibiotic cocktail consisting of Ampicillin, Metronidazole, Neomycin, and Vancomycin demonstrated beneficial effects, including improvements in cardiovascular damage and amelioration of IxS levels. Subsequently, we investigated IxS-mediated inflammation in vitro by focusing on the AhR. Pharmacological AhR inhibition using BAY 2416964 demonstrated a dose-dependent decrease of IxS-induced expression of AhR target genes, such as CYP1a1, in murine splenocytes. Furthermore, BAY 2416964 exhibited an attenuating effect on T_H17 cell polarization – a pro-inflammatory T cell subset shown to of relevance in CKD. Building upon these findings, our ongoing experiments aim to elucidate the impact of BAY 2416964 on the development of CKD and CVD in our CKD mouse model.

Taken together, microbial metabolites contribute to the development of CVD in CKD, presumably by stimulating pro-inflammatory effects in an AhR-dependent manner. Considering AhR antagonistic strategies might prove of value in preventing CKD-related comorbidities.

[P21] Modulation of AhR activity by tryptamine-based compounds as a tool to abrogate inflammatory cues in intestinal epithelial cells

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The mucosal intestinal epithelial barrier represents a physical barrier shielding our body from many harmful agents and substances. The Intestinal epithelial cells that constitute this barrier are exposed to AhR and/or pregnane X receptor (PXR) ligands of dietary origin or, produced by intestinal microbiota. In recent years, in particular the tryptophan-derived AhR ligands produced by gut microbiota have gained significant attention as regulators of intestinal barrier homeostasis. Here, we studied the effects of chemically modified tryptamines targeting AhR and/or PXR, and we assessed their anti-inflammatory and epithelial barrier protective activities in human colon cancer-derived epithelial cell models, HT-29 and Caco-2 cells. We found that selected tryptamine derivatives prevented the upregulation of pro-inflammatory chemokines (CXCL-8 and MCP-1) in cells exposed to a mixture of inflammatory cytokines. In line with this, these tryptamines also prevented the downregulation of tight junction proteins, such as occludin and TJP-1/ZO-1, at mRNA and protein levels. We also observed prevention of inflammation-induced disruption of transepithelial resistance and barrier permeability. Presently, we are evaluating the functional role of AhR in these effects using AhR knockout models, as well as *in vivo* efficiency of the selected tryptamine derivatives. Our data so far indicate that specific tryptamine derivatives may decrease inflammatory damage elicited by inflammatory cytokines through the activation of AhR-related signaling pathways, but that also a combined action of these substances on both AhR and PXR should be taken into consideration, when designing active compounds. Funded by the Czech Science Foundation, project no. 22-00355S. G. V.-G. is funded by Secretaría de Educación, Ciencia, Tecnología e Innovación de la Ciudad de México, Grant No. SECTEI/102/2022.

[P22] Aryl hydrocarbon receptor confers protection against macrophage pyroptosis and intestinal inflammation through regulating polyamine biosynthesis

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Aryl hydrocarbon receptor (AhR) functions in inflammation regulation, but the underlying mechanism remains unclear. Here we show that AhR deficiency exacerbated intestinal inflammation by inducing an immune cell population shift, with a significant increase in the monocyte-macrophage lineage. Mice with AhR deficiency in myeloid cells（$AhR^{\triangle mye}$） developed more severe dextran sulfate sodium (DSS)-induced colitis, with concomitant increased macrophage pyroptosis. Dietary supplementation with the AhR pro-ligand, indole-3-carbinol, ameliorated DSS-induced colitis in $AhR^{fl/fl}$, but not in $AhR^{\triangle mye}$ mice. Mechanistically, AhR signaling inhibited macrophage pyroptosis by promoting ornithine decarboxylase 1 (Odc1) transcription, to enhance polyamine biosynthesis. The increased polyamine (particularly spermine) inhibited NLRP3 inflammasome assembly and subsequent pyroptosis by suppressing K^+ efflux. Furthermore, AHR expression was positively correlated with ODC1 in intestinal mucosal biopsies from patients with ulcerative colitis. Thus, we propose a functional role for the AhR/ODC1/polyamine axis in regulating intestinal homeostasis, providing new targets for treatment of inflammatory diseases.

[P23] Modulation of the Molecular Expression and Function of BCL-2 Family Proteins by Aryl Hydrocarbon Receptor in Breast Cancer Stem Cells

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Breast cancer (BC) is a frequently diagnosed neoplasm in women and the second major cause of cancer-related deaths. Despite the remarkable advancements in understanding BC and its treatments, the diseases still pose numerous challenges. Many BC patients develop metastasis and advanced tumors, increasing morbidity and mortality. There is substantial evidence that tumor relapse in BC patients is driven by a unique population of cells called cancer stem cells (CSCs). Breast CSCs confer stemness to BC and survive through the maintenance of several mechanisms, among which the involvement of the aryl hydrocarbon receptor (AhR) has recently been evaluated. However, the correlation of this receptor with the chemoresistance-mediated BCL-2 family pathway for the development of breast CSCs remains unclear. This study evaluates the correlation between AhR and BCL-2 proteins *in vitro* and patient breast tissue samples. Breast CSCs were enriched through mammosphere culture and characterized by flow cytometry. BC cells and breast CSCs were exposed to AHR or BCL-2 modulators to determine the expression of different genes through RT-PCR, Western blot analysis, immunofluorescence, and flow cytometry. The expression of AhR and BCL-2 in human breast tissues was determined by IHC. Our results showed that AhR, CYP1B1, and BCL-2 proteins' constitutive expression was significantly higher in mammospheres than in differentiated cancer cells. Moreover, AhR induction using DMBA or inhibition using α-NF proportionally modulated the expression of BCL-2 proteins, and inhibition of BCL-2 inhibited the expression of AhR and its regulated genes. The expressions of AhR and BCL-2 were also found to be higher in tissues of BC patients compared to the non-cancerous BC tissues. These findings demonstrated crosstalk between AhR and BCL-2 for developing breast CSCs, which offers a solid basis for designing therapeutic strategies to target AhR to overcome drug resistance in BC.

[P24] Investigating the role of AHR ligands in different cancer entities

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The Aryl hydrocarbon receptor (AHR) is a transcription factor that is associated with the metabolism of different compounds, development, immune responses and cancer. The AHR can act both as a tumor suppressor and encourage tumor progression in different cancer types, in a context specific manner. Until now, the instances where it drives or suppresses cancer progression are poorly understood. AHR can bind to various metabolites, which allows its activity as a transcription factor of different target genes. Furthermore, it has been previously shown that the AHR can interact with different partners, with some of which it shares chemical and biological similarities, all of which are suspected to have a role in its signaling.

It has been well established that the AHR can bind not only to xenobiotic chemicals, but also to tryptophan metabolites. Our aim is therefore to further investigate additional ligands that can trigger activation of the AHR and to elucidate the underlying mechanisms that orchestrate AHR transcriptional activity. To do so, we are using different cancer entities with varying expression profiles of the AHR and its partners.

[P25] Dissecting the role of the kynurenine pathway and aryl hydrocarbon receptor in Ewing sarcoma

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Ewing sarcoma (EwS) is an aggressive, immunologically rather 'cold', pediatric cancer driven by a single dominant driver mutation – the chimeric *EWSR1::FLI1 (EF1*) fusion transcription factor, whose expression and thus activity undulates in EwS cells. While EF1-high cells are more sessile and proliferative, EF1-low cells gain invasiveness. We hypothesized that EF1 modulates the expression of key metabolic enzymes promoting tumorigenesis and metastasis. To test this possibility, we follow two orthogonal strategies: A) an agnostic analysis of EF1-high/low cells using single-cell and spatial metabolomics, and B) a hypothesis-driven approach based on data for a prominent role of EF1 in rewiring kynurenine metabolism. To that end, we established 3D cell culture conditions in human plasma-like medium (HPLM) and generated EwS cell lines with a highly dynamic fluorescent reporter (d2EGFP) induced by EF1, which enables us to monitor the EF1 transcriptional activity at the single-cell level. Preliminary results demonstrated that low EF1 expression is associated with increased production of kynurenine from tryptophan likely through modulation of TDO2 (tryptophan 2,3-dioxygenase), IL4I1 (interleukin 4 induced 1) and KMO (kynurenine 3-monooxygenase) expression levels. Analysis of gene expression data from patients showed, that low EF1-levels are correlated with higher activity of the pro-tumorigenic and immune-suppressive aryl hydrocarbon receptor (AHR) – a major receptor for kynurenine – which is linked to worse overall survival in EwS. In addition, the conditional knockdown of AHR in EwS cells led to a significant decrease in cell proliferation and clonogenicity. Experiments are ongoing to further decipher how EF1 modulates kynurenine/AHR signaling and whether this is linked to metastasis and immunological responses. We anticipate that a better understanding of the metabolic landscape in EwS will yield new therapeutic options.

[P26] Novel insights into the oncogenic role of AHR in high-risk breast cancers

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Phenotypic plasticity is the ability of an organism to respond and adapt to different environmental insults. The Aryl Hydrocarbon Receptor (AHR), a ligand-activated transcription factor, can sense environmental clues and drive phenotypic plasticity. Once activated, AHR shuttles to the nucleus where it transcribes hundreds of genes involved in metabolism, detoxification, cell survival, cell differentiation and immune responses. Due to its diverse pro-survival functions, AHR has been found to be overexpressed in many cancer types, thereby representing an attractive therapeutic target to hijack multiple cancer dependencies at once. We have previously shown that AHR sustains the growth of high-risk breast cancers, such as the triple-negative/BRCA1-associated subtype. In these tumors, AHR has a dual function: to maintain the survival of cancer cells and to regulate their communication with the surrounding microenvironment. As a result, AHR exerts potent cell-intrinsic and cell-extrinsic oncogenic functions, providing the rationale for the ongoing first-in-human clinical trials with AHR inhibitors. Here we will present the generation and functional characterization of novel cellular and animal models to study AHR activities in breast cancer. We are particularly interested in two aspects of AHR: 1) its ligand sensing ability within the PAS-B domain and 2) its role in the tumor microenvironment. To address the first aspect, we have generated a set of human breast cancer cell lines harbouring the complete gene deletion of AHR, a form of AHR lacking the PAS-B domain or carrying mutations in each of the three cysteine residues contained in the PAS-B domain. Through the analysis of AHR interactome and transcriptome in these cells, we shed light on novel AHR-regulated pathways as well as the role of cysteine residues in controlling AHR activity. To study the immune-related role of AHR, we have generated a mouse model expressing a “trackable” form of AHR, through the expression of the mCherry fluorescent tag. Preliminary transplantation studies in this model show that tumor-supporting macrophages which invade BRCA1/p53 deficient mouse mammary tumors strongly express AHR levels. A plan to use this model for a barcoded, single cell-based, functional screen will be presented.

[P27] Tumor-Suppressive Functions of the Aryl Hydrocarbon Receptor

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There are conflicting reports describing the role of the aryl hydrocarbon receptor (AhR) in cancer. AhR-knockout mice do not develop tumors spontaneously, yet the AhR can act as a tumor suppressor in certain contexts. We investigated the role of AhR both in vivo and in cell-based models. Loss of tumor suppression by p53 is common in human cancer. To investigate AhR function in the absence of p53, we generated mice lacking both AhR and p53. Mice deficient for AhR and p53 had shortened lifespan, increased tumorigenesis, and an altered tumor spectrum relative to control mice lacking only p53. We also investigated endogenous role of the AhR in cell based models and discovered its ability to suppress cancer cell aggressiveness. Different small-molecule AhR ligands drive strikingly different cellular and organismal responses. I will summerize our investigations that have led to the identification of new small molecule activators of the AhR with anti-cancer actions and delineation of their molecular mechanims of action.

[P28] Loss of AHR in CR705 pancreatic cancer cells reduces tumor development due to increased anti-tumor immune responses

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and deadly forms of cancer due to late diagnosis and lack of treatment options. One promising therapeutic strategy is to increase immune cell infiltration to the tumor microenvironment. The aryl hydrocarbon receptor (AHR) is an essential gatekeeper integrating metabolic signals to promote immunosuppression, regulate effector immune cells and enhance tumorigenesis. AHR's immunosuppressive actions allow tumor cells to "hide" from immunosurveillance. To investigate whether loss of AHR protects against PDAC, we used the murine pancreatic cancer cell line, CR705, and generated CR705 Ahr^{KO} cells using CRISPR/Cas9. Loss of AHR did not affect cell proliferation compared with WT. Consistent with these findings, WT and Ahr^{KO} CR705 cells were injected subcutaneous in immune incompetent NOD.Cg-$Prkdc^{scid}$ $Il2rg^{tm1Wjl}$/SzJ (NSG) mice or immune competent C57BL/6J mice. We did not observe any differences in tumor development between injection of WT cells compared with Ahr^{KO} CR705 cells into NSG mice. However, when these cell lines were injection into C57BL/6J mice, tumors from CR705 Ahr^{KO} cells developed significantly slower than tumors from CR705 WT cells. Histological analyses revealed an increased immune cell infiltration, including $CD3^+$ and $CD8^+$ T-cells, and macrophages. RNA sequencing of the tumors resulted in 1091 genes upregulated and 576 genes downregulated in Ahr^{KO} tumors compared with WT tumors. Pathway analysis revealed that T cell activation, adaptive immune response and lymphocyte mediated immunity were among the top 5 upregulated pathways. Collectively, these results of this study support the idea of targeting AHR as a therapeutic strategy against PDAC.

[P29] Serum tryptophan metabolites mediate constitutive AHR activity in head and neck squamous cell carcinoma cells

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High level of AHR protein and activation has been associated with aggressive tumor phenotypes and migratory potential in head and neck squamous cell carcinoma (HNSCC). Compounds derived from host tryptophan metabolism (e.g., kynurenine, indole-3-acetate) and from bacterial tryptophan metabolism (e.g., indole-3-propionate) can activate AHR. A fundamental question addressed here is whether these tryptophan (Tryp) metabolites can mediate persistent AHR activation within tumor tissues and alter tumorigenesis. Our research work aims to determine whether the continuous exposure of Tryp metabolites at physiologically relevant concentrations leads to sustained AHR activation in HNSCC cells and its subsequent effect on tumor cell phenotypes. Here, we present data demonstrating sustained AHR activation by Tryp metabolites in two HNSCC cell lines. A total of 6 Tryp metabolites present at significant levels in serum with known AHR agonist activity were identified and quantified from 40 healthy individuals on a controlled diet by LC/MS/MS. HN30 and OSC19 cells were treated with a representative pool of 6 Tryp metabolites in a cell culture system to achieve continuous exposure to the Tryp metabolites, thus mimicking blood circulation in vivo. Continuous exposure of Tryp metabolites resulted in sustained AHR activation in HN30 and OSC19 cells, confirmed by induced mRNA expression of target genes and subsequent protein levels at six different time points. Immunocytochemical localization of the AHR after treatment with Tryp metabolites displayed enhanced nuclear translocation, further confirming that Tryp metabolites mediate AHR activation. LC/MS/MS data revealed the presence of Tryp metabolites inside cells starting from 2h post-treatment to the last time point 24h. Cell viability assays indicated no cytotoxic effects of Tryp metabolites on HNSCC cells. RNA seq analysis on cells exposed to Tryp metabolites for 72h showed upregulation of known AHR target genes as well as genes for cytokines involved in tumor-immune microenvironment modulation. The colony formation assay revealed an increased number of colonies formed upon Tryp metabolites treatment, indicating increased survival and proliferative potential. Overall, our work has demonstrated that sustained AHR activation in HNSCC cells upon continuous exposure to Tryp metabolites, supporting the concept that endogenous Tryp metabolites in HNSCC patients would impact tumor progression, metastasis, and treatment outcomes.

[P30] Metabolic transitions driving trajectories of AHR activation profiles in cancer

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Cancers that activate the aryl hydrocarbon receptor (AHR) are more aggressive as a result of the AHR mediated dampening of the immune response and increase in cell motility and migration. The AHR is a transcription factor that translocates to the nucleus upon binding of ligands. The effects mediated by AHR activity are ligand and cell type specific. The sources of endogenous ligands that activate AHR can come from the microbiota as well as the catabolism of tryptophan (Trp) by Trp catabolizing enzymes (TCEs) such as indoleamine-2,3-dioxygenase 1 (IDO1) and tryptophan-2,3-dioxygenase 2 (TDO2) that produce kynurenine (Kyn), and interleukin-4 induced 1 (IL4I1) that produces indole-3-pyruvate (I3P). Immune checkpoint inhibitors (ICIs) have shown great success in some cancer patients. ICIs lead to the activation of the immune system, which in turn becomes more capable to clear the tumor. However, ICIs upregulate TCEs leading to AHR activation and subsequent immune suppression. Combining ICIs with IDO1 inhibitors was thought to overcome this AHR mediated secondary resistance, but clinical trials in phase III failed to meet the primary endpoint of progression free survival in comparison to standard of care. Using machine learning we were able to predict the production of endogenous ligands that can activate the AHR in multiple cancers. Graph embedding of cancer patients reveals a clear separation of AHR active and inactive patients. Furthermore, the embedding indicated a clear trajectory of cancer patients with different AHR activity profiles. Our data show an alignment of different clinical subgroups with AHR profile trajectories, and that within a clinical subgroup metabolic transitions marked by various endogenous ligand producing enzymes lead to distinct AHR profiles that could influence the choice of treatment, dosing, and decisions on treatment change.

[P31] Identification of downstream effectors of AHR activation in glioblastoma

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The aryl hydrocarbon receptor (AHR) is a ligand-activated protein that acts as a transcription factor, integrating signals from internal and external stimuli. The L-amino acid oxidase interleukin-4-induced 1 (IL4I1) catabolizes tryptophan to indole-3-pyruate, which further breaks down into potent AHR ligands, including indole-3-aldehyde and kynurenic acid. Overexpression of IL4I1 AHR-dependently increases the malignant properties of glioblastoma cells including their motility. Analysis of RNAseq data identified several candidate genes regulated by IL4I1 and its metabolites in an AHR-dependent manner, which may be involved in promoting malignant effects mediated through the AHR. We experimentally validated whether the AHR indeed regulates these candidates. Our results reveal potential downstream effectors of the AHR that may represent drug targets for glioblastoma therapy.

[P32] Transcriptomic analysis reveals that canonical aryl hydrocarbon receptor (AhR) pathway activation correlates with improved clinical outcome of lung adenocarcinomas patients

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Lung carcinomas remains the foremost cause of cancer-related fatalities globally. Within this context, lung adenocarcinomas (ADC), the predominant histological subtype, exhibit elevated AhR expression levels compared to normal lung tissue. Furthermore, a strong correlation exists between the copy number variation (CNV) of the AhR gene and its expression at mRNA level. Our transcriptomic meta-analysis of RNA-seq data from 420 ADC patients (TCGA Research Network) revealed distinct patients clusters based on AhR and ARNT expression, both players of the canonical AhR pathway. High co-expression of AhR and ARNT correlates with improved overall survival, suggesting the activation of canonical pathway. This highlights the potential of new methodologies for transcriptomic data analyses to identify novel prognostic and therapeutic approaches for lung ADC patients.

[P33] PDAC aggressiveness could be attributed to PTGS2 and EMT genes through upregulation of AHR

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AHR is a transcription factor that is commonly upregulated in pancreatic ductal adenocarcinoma (PDAC). AHR overexpression in linked to increased tumour cell migration, invasion, cancer progression and aggressiveness. Fast PDAC progression and metastasation is a major burden in PDAC which hinders its treatment and lowers survival. Cell metastasation and migration is attributed to epidermal-mesenchymal transition (EMT) and proteins such as PTGS2, however the role of AHR in EMT or overall cell migration of PDAC is still unclear. With upregulated AHR expression the cells become more aggressive and motile and by modulating AHR in PDAC cells it could be possible to lower their migratory capabilities. We hypothesize that AHR is connected to EMT and PTGS2 pathways and by targeting its synthesis cell migration can be reduced.

PDAC cell line (BxPC-3) was used for experiments. *AHR* gene was silenced by siRNA transfection or completely knocked-out (KO) by CRISPR-CAS9 gene. Afterwards the cells were seeded into 24-well plates for scratch assay to determine migration. After 24 hours of seeding, a wound (scratch) was inflicted with a pipette tip and the cell growth medium was changed into medium without serum. The wells were photographed after making the wound and after 24 hours of incubation. The influence of silencing on RNA expression and RNA expression of PDAC patient tumour samples (N=37) were analysed by qRT-PCR

The results of PDAC patient tumour samples showed a strong positive correlation between the expression of AHR and PTGS2 or EMT genes such ZEB1, SNAIL1, SNAIL2. A decrease in PTGS2 and EMT genes (ZEB1, ZEB2, SNAIL2, TWIST) was also seen when silencing AHR in PDAC cells. The results of migration assay showed that the untreated cells and KO vector control cells almost fully covers the inflicted wound after 24-hours of infliction. Silencing of AHR gene caused the cells to migrate slower and cover only 36 % (+/- 12%) after 24 hours. Moreover, AHR KO covered only 37 %(+/- 23%) of the wound after 24 hours.

In conclusion, lowering AHR expression reduces cell migratory capabilities. Silencing of AHR reduced the expression of PTGS2 and EMT genes. Moreover, AHR expression correlates with PTGS2 and EMT gene expression in patient samples. These findings suggest that PDAC aggressiveness and migration could be attributed to PTGS2 and EMT genes through upregulation of AHR and it could be reduced by targeting AHR expression.

[P34] A Comprehensive Proteomic Analysis of the Role of the Aryl Hydrocarbon Receptor in Ovarian Cancer Stemness and Metastasis

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Gynecological malignancies pose a severe threat to female lives. Ovarian cancer (OC), the most lethal gynecological malignancy, is clinically presented with chemoresistance and a higher relapse rate. Several studies have highly correlated the incidence of OC to exposure to environmental pollutants, such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a process mainly mediated through activating the aryl hydrocarbon receptor (AhR). We have previously reported that exposure of ovarian cancer cells to TCDD, an AhR activator, significantly modulated the expression of several genes that play roles in stemness and chemoresistance. However, the effect of AhR activation on the whole ovarian cancer cell proteome aiming at identifying novel druggable targets for both prevention and treatment intervention purposes remains unrevealed. For this purpose, we conducted a comparative proteomic analysis of OC cells A2780 untreated/treated with TCDD for 24h using a mass spectrometry-based label-free shotgun proteomics approach, and the most significantly dysregulated proteins were validated by Western blot analysis. Our results showed that upon AhR activation by TCDD, we found that out of 2598 proteins identified, 795 proteins were upregulated, and 611 were downregulated. String interaction analysis and KEGG-Reactome pathway analysis approaches identified several significantly dysregulated proteins that were categorized to be involved in chemoresistance, cancer progression, invasion and metastasis, apoptosis inhibition, survival, and worse prognosis in OC. Our study helped us to identify the cross-talk between AhR and several other molecular signaling pathways and their involvement in the carcinogenesis and chemoresistance of OC. In conclusion, this study provides a better idea about the role and involvement of AhR in ovarian carcinogenesis and chemoresistance. Moreover, the study suggests that AhR is a potential therapeutic target for OC prevention and maintenance.

[P35] Interplay of Aryl Hydrocarbon Receptor (AHR) Activation and NAD Metabolism in Glioblastoma

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Glioblastoma (GB), renowned for its aggressive nature, necessitates a comprehensive exploration of cellular intricacies for targeted therapeutic strategies. This study explores the interplay between aryl hydrocarbon receptor (AHR) activity and nicotinamide adenine dinucleotide (NAD) metabolism in GB cells. NAD serves as a pivotal regulator in crucial biochemical processes, including redox reactions and cellular growth, with its production occurring through three distinct pathways originating from different precursors—tryptophan, nicotinic acid and nicotinamide. In GB, tryptophan-2,3-dioxygenase (TDO2), a key enzyme degrading Trp, plays a role in enhancing tumor cell motility and inhibiting anti-tumor immune responses, underscoring its significance in cancer biology. Preliminary evidence in GB cells indicates that AHR modulation affects cellular viability, prompting an in-depth investigation into potential connections with NAD dynamics. By conducting comprehensive omic analyses, encompassing metabolomics, proteomics, and transcriptomics, we aim to uncover the nuanced molecular mechanisms governing the interplay between AHR activation and NAD metabolism in GB. These analyses are expected to contribute to a broader understanding of the coordinated roles of AHR activity and NAD metabolism in cellular homeostasis and pathology.

Keywords: Tryptophan metabolism, glioblastoma, NAD metabolism, omics

[P36] Preclinical characterization of SZDM-01, a novel inhibitor of AhR with potent anti-tumor activity

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The aryl hydrocarbon receptor (AhR) is a transcription factor, activated by structurally diverse compounds from the environment, diet, microbiome, and host metabolism. Emerging evidences suggest that AhR is a key regulator maintaining immune homeostasis in response to environmental cues, and changes in AhR activity have been associated with immune disorders and cancer. AhR activation by tryptophan metabolites plays a pivotal role in shaping a suppressive immune microenvironment during carcinogenesis and metastatic process, making AhR an promising target for anti-cancer immunotherapy. Here we report the results with a small molecule, SZDM-01, an AhR ligand which exerts a potent anti-cancer effect via inhibition of AhR pathway. In dioxin response elements (DRE)-driven reporter assays, SZDM-01 inhibited reporter expression induced by AhR agonists such as TCDD or kynurenic acid with high potency in stable cell lines including HepG2、Hepa1-6 and H4IIE. SZDM-01 dose-dependently reduced EC_{50} of TCDD/ITE, suggesting SZDM-01 directly competed the binding sites of these orthodox exogenous/endogenous AhR ligands. Moreover, SZDM-01 dose-dependently inhibited TCDD induced AhR nuclear translocation, and accordingly expression of downstream genes such as CYP1A1. These data suggested SZDM-01 was indeed a potent (HepG2 cell line: IC50 = 0.25 nM) AhR antagonist. In PK studies, SZDM-01 showed good orally bioavailability (F = 59.1%) in rat. Tumor growth inhibition is observed with SZDM-01 in syngeneic CT26 model etc. SZDM-01 has a favorable safety profile and is currently under investigation in preclinical studies.

[P37] Unlocking Nature's Code: A Comprehensive Exploration of AHR Modulators for Therapeutic Discovery

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The aryl hydrocarbon receptor (AHR) has gained strong traction in recent years as a therapeutic target for its ubiquitous expression, promiscuity in modulator binding, and capacity to act as a mediator for many biological functions including inflammation, immune cell development, and regulation of antitumor activity for cancer treatment. Exogenous and endogenous AHR modulators have incredible structural diversity, and these variations underlie a wide range of agonist or antagonist responses. However, previous studies have suggested that even seemingly minor alterations in structural arrangement can drastically alter AHR modulating activity. However, understanding the physio-chemical properties corresponding to the ligands' functional potency have yet to be identified, and understanding the molecular characteristics that favour AHR activation or antagonism would enable more efficient identification of relevant metabolites that could serve as preventative measures or interventions. Natural product derived AHR modulators are found to play a key role in regulating inflammatory conditions through diet and could serve as a more accessible, affordable, and high adherence treatment. Importantly, several culinary and medicinal mushrooms including *Agaricus bisporus*, *Pleurotus ostreatus, Lentinula edodes, Hypsizygus tessulatus,* and *Ganoderma lucidum* have already shown AHR modulating activity, with some containing isomers with drastically different agonistic activity on their own. This study employed a multidisciplinary approach: leveraging the power of multivariate machine learning models, we aim to discern patterns within chemical descriptors, using docking data derived from the recently elucidated cryo-electron microscopy (cryo-EM) structure of AHR, and biological assay data to guide relevant characteristic identificaiton. By amalgamating these diverse datasets, we can identify distinct properties that characterize potent AHR modulators. This in silico approach is part of a comprehensive platform for identifying, characterizing, and optimizing novel AHR modulators from natural sources. This research not only contributes to the expansion of our pharmacological toolkit but also underscores the potential of natural products in fostering innovative therapeutic interventions targeting the AHR pathway with broad implications for human health.

[P38] Good or bad? Consequences of aryl hydrocarbon receptor activation in intestinal injury and regeneration

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The intestinal epithelium is constantly exposed to a wide variety of dietary-derived and microbial metabolites, and chemical pollutants (xenobiotics), many of which can be sensed by the transcription factor aryl hydrocarbon receptor (AHR). AHR activation by natural ligands promotes intestinal barrier integrity by inducing the production of barrier protective cytokines such as IL-22 and by regulating the differentiation of intestinal stem cells. Conversely, it is generally accepted that prolonged AHR activation by xenobiotics disrupts physiological processes by a molecular mechanism yet to be defined. Here, we are investigating whether and how exposure to xenobiotics ligands interferes with known AHR physiological functions in the intestine.

Exposure to a single dose of 2,3,7,8-Tetrachlorodibenzo-P-dioxin (TCDD), an AHR xenobiotic ligand, resulted in sustained receptor activation across different organs, without causing major changes in immune cell populations in the colon under steady state conditions. Mice exposed to TCDD prior to *Citrobacter rodentium* infection, an *in vivo* model of intestinal injury and regeneration, showed prolonged persistence of the pathogen and defective immune activation. Exposure to natural and xenobiotic AHR ligands in mice with conditional genetic alterations in the AHR signalling pathway will allow us to identify the cell type responsible for the delayed pathogen clearance.

The focus of this ongoing study is to identify the underlying mechanism for the delayed clearance and to address the effect of sustained AHR activation in different cell types in models of intestinal injury and regeneration.

[P39] A Novel AhR Inhibitor TM442 Targeting Atopic Dermatitis

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The onset and exacerbation of atopic dermatitis (AD) are linked to exposure to environmental pollutants. The Aryl hydrocarbon Receptor (AhR) functions as a sensor of environmental pollutants, regulating genes encoding detoxication enzymes such as *CYP1A1*. In previous studies, we established transgenic mouse lines expressing a constitutively active form of AhR (AhR-CA) in the epidermis, mimicking chronic exposure to pollutants on the skin. AhR-CA mice displayed AD-like symptoms, including itching, allergic inflammation, and barrier dysfunction. In the skin of AhR-CA mice, AhR directly enhances a neurotrophic factor called Artemin, leading to increased hypersensitivity to itch. Similar symptoms were observed in wildtype mice after prolonged application of an AhR agonist, 7,12-dimethylbenz(a)anthracene (DMBA), to the skin. This suggests that prolonged AhR activation in the epidermis triggers the development of AD, indicating AhR as a potential target for AD treatment. To develop potent clinical AhR inhibitors, we screened compounds from the Tohoku University chemical library that exhibit inhibitory effect on the AhR activation induced by an AhR agonist, 3-methylcholanthrene (3-MC) using a Cyp1-luciferase (Luc) reporter assay system. Several compounds isolated from this library showed promising results by reducing Luc activity when applied to stably transformed Cyp1-Luc-HaCaT cells. Among these compounds, we selected TM442 as the lead compound for potent AhR inhibitor. TM442 reduced AhR-target gene expression induced by AhR agonists both *in vitro* and *in vivo*. Moreover, TM442 blocked 3MC-induced nuclear accumulation of AhR protein, indicating a direct inhibition of AhR activity. Topical application of TM442 markedly alleviated the severity of dermatitis and reduced scratching behavior in AhR-CA mice. Consistent with these findings, TM442 suppressed AD-like symptoms induced by exposure to DMBA on the skin. These results underscore the efficacy of TM442 in the AhR-activated AD models, suggesting that TM442 represents a promising therapeutic agent for AhR-activated AD patients.

[P40] Aryl hydrocarbon receptor restricts neuroregeneration and repair of the injured peripheral nervous system

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Injured neurons sense environmental cues to balance neural protection and axon regeneration, but the mechanisms are unclear. Here, we unveil aryl hydrocarbon receptor (AhR), a ligand-activated basic helix-loop-helix/PER-ARNT-SIM (bHLH-PAS) transcription factor, as molecular sensor and key regulator of acute stress responses at the expense of axon regeneration. We demonstrate responsiveness of dorsal root ganglia (DRG) sensory neurons to ligand-mediated AhR signaling, which functions to inhibit axon regeneration. *Ahr* deletion mimics the conditioning lesion in priming DRGs to initiate axonogenesis gene programs; upon peripheral axotomy, *Ahr* ablation suppresses inflammation and stress signaling while augmenting pro-growth pathways. Moreover, comparative transcriptomics revealed signaling interactions between AhR and HIF-1α, two structurally related bHLH-PAS α units that share the dimerization partner Arnt/HIF-1β. Functional assays showed that the growth advantage of AhR-deficient DRG neurons requires HIF-1α; but in the absence of Arnt, DRG neurons can still mount a regenerative response. We further unveil a link between bHLH-PAS transcription factors and DNA hydroxymethylation in response to peripheral axotomy, while neuronal single cell RNA-seq analysis revealed a link of the AhR regulon to RNA polymerase III regulation and integrated stress response (ISR). Altogether, AhR activation favors stress coping and inflammation at the expense of axon regeneration; targeting AhR can enhance nerve repair.

Key words:
Aryl hydrocarbon receptor (AhR), Axon regeneration, Conditioning lesion of DRG, Hypoxia-inducible factor (HIF), Arnt, DNA hydroxymethylation, Integrated stress response (ISR)

[P41] A1007, a novel gut-enriched AhR modulator for the treatment of Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a condition characterized by chronic inflammation and impaired barrier function of the gastrointestinal tract, manifesting itself either as Crohn's disease (CD) or ulcerative colitis (UC). Over the past few decades, the emergence of advanced therapies (e.g. several Biologics, JAK inhibitors) has revolutionized the treatment of IBD. However, the limitations of these therapies leave an unmet medical need for safer, more effective, or convenient treatment options. Recent publications have demonstrated that AhR activation will regulate immune responses and alleviate symptoms of IBD. In this study, a series of novel small molecule AhR agonists have been identified and profiled in several disease relevant biochemical, pharmacological, and safety assays.

Our most advanced compound, A1007 has overall good biophysical and ADME properties providing oral bioavailability and gut-enriched tissue distribution. A1007 has multiple pharmacological MOAs showing reduction of pro-inflammation cytokines in human primary cells, increase of Treg cells, and increase in tight junction proteins in epithelial cells. In several mouse IBD models (e.g. TNBS model), A1007 shows clear dose response efficacy superior or comparable to several SOCs. A1007 significantly reduces the DAI score, increases body weight, improves colon length & weight, reduces colon histopathology score, repairs gut permeability and integrity, and rebalances cytokine profiles and immune homeostasis in TNBS model.

In summary, A1007 is a novel orally bio-available gut-enriched small molecule AhR agonist with unique *in vitro* pharmacology properties and *in vivo* efficacy. Our results underscored the great therapeutic potential of A1007 for the treatment of IBD.

[P42] AHR-driven transcriptional alterations in liver cell types in response to acute TCDD exposure

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a persistent environmental contaminant that disrupts hepatic gene expression and function mediated by the aryl hydrocarbon receptor (AHR) resulting in hepatotoxicity and non-alcoholic fatty liver disease (NAFLD)-like pathologies, that includes the progression of steatosis to steatohepatitis with fibrosis. Although diverse liver cell types are involved, the role of specific cell types has not been thoroughly investigated. This study examined the time-dependent gene expression effects in specific liver cell types following oral gavage with 30 μg/kg of TCDD or sesame oil and sacrificed at 2, 4, 8, 12, 18, 24, or 72 hours in male C57BL/6 mice. Leiden clustering and manual annotation using known marker genes identified 10 major liver cell types, as well as 2 macrophage and 8 endothelial cell (EC) subtypes. While most cell type populations remained relatively constant, neutrophils increase at 72 hours in response to treatment. Known AHR battery genes (e.g. *Cyp1a1*, *Ahrr*, *Tiparp*, *Nqo1*) showed time- and cell type-dependent induction, with hepatocytes being the only cell type to show differential expression across all time points. Hepatocytes, ECs, and hepatic stellate cells (HSCs) were the only cell types with cell-specific gene expression changes with known AHR binding sites (1,550, 102, and 40, respectively). Functional enrichment analysis in response to TCDD revealed disruptions in the metabolism of steroids, primary bile acids, vitamins, retinols, and glutathione in addition to one-carbon metabolism. EC differential gene expression was associated with PI3-AKT signaling, as well as protein processing and transport pathways. HSCs were enriched in protein processing, ABC transporters, and the dysregulation of tryptophan and linoleic acid metabolism. These results suggest that early responses to TCDD trigger cell-specific gene expression changes, contributing to hepatotoxicity and the emergence of hepatic pathologies related to NAFLD. GNC was supported by T32ES007255. This project is funded by R01ES029541 and the SRP P42ES004911.

[P43] Impact of polycyclic aromatic hydrocarbons and skin sensitizing substances on the oxidative stress response of keratinocytes

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Contact allergies are prevalent in up to 20 % of the general population and are caused by repeated skin exposure to skin sensitizing substances, including various metals, fragrances, preservatives and dyes. These may lead to the development of an allergic contact dermatitis, a common inflammatory skin disease. Studies indicate the generation of reactive oxygen species (ROS) and activation of cytoprotective NRF2 signaling by skin sensitizing substances as a key mediator of chemical-induced contact allergies. Exposure to ubiquitous environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs) also generate ROS in vitro by activating the aryl hydrocarbon receptor (AHR), inducing cytochrome P450 (CYP) 1-isoforms and undergoing aldo-keto reductase (AKR) 1-dependent redox cycling.

This study aimed to investigate the modulation of the oxidative stress response in human HaCaT keratinocytes when co-exposed to established skin sensitizing substances and exposure-relevant PAHs such as benzo[a]pyrene or benzo[k]fluoranthene. In addition to a synergistic increase in heme oxygenase 1 (HMOX1) expression, a marker for activation of the NRF2 antioxidant pathway, ROS analyses confirmed that the substances potentiate each other´s effect. Interestingly, this effect was abolished in CRISPR/Cas-mutated keratinocytes lacking functional expression of CYP1A1 and AKR1C3, respectively. Further analyses revealed that elevated ROS formation because of co-exposure is based on AHR-mediated expression of CYP1A1 and AKR1C3 as well as NRF2-dependent AKR1C3-expression by skin sensitizing substances. Although expression of CYP1A1 is crucial for activation of PAHs, these findings indicate that increased ROS formation due to co-exposure is mainly mediated by additional induction of AKR1C3 and associated redox cycling of PAHs. Furthermore, this study suggests that the induction of AKR1C3 expression depends not only on the sensitizing potency but also on the reaction mechanism of these substances, particularly on potent Michael acceptors.

These results demonstrate potential risks arising from co-exposure of sensitizing substances with PAHs. The significant increase in ROS formation carries toxicological implications, while synergistic activation of the NRF2 signaling pathway might increase the sensitizing potential of contact allergens. Regarding exposure-relevant environmental pollutants, these data could enhance risk assessment of sensitizing substances.

[P44] Comparison of transcriptomic signatures in murine macrophages induced by toxic (benzo[*a*]pyrene) and non-toxic (indol-3-carbinol) AHR ligands

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that regulates a broad range of target genes involved in the xenobiotic response, cell cycle control and circadian rhythm. AHR is constitutively expressed in macrophages (Mph), acting as key regulator of cytokine production. While proinflammatory cytokines, i.e., IL-1β, IL-6, IL-12, are suppressed through AHR activation, anti-inflammatory IL-10 is induced. However, the underlying mechanisms of those effects, the importance of the specific ligand structure and differences between toxic and non-toxic AHR ligands are not yet completely understood. Therefore, in this study we have compared the global gene expression pattern in activated murine bone marrow-derived macrophages (BMMs) subsequently to exposure with either benzo[*a*]pyrene (BaP) or indole-3-carbinol (I3C), representing a high-affinity xenobiotic vs. a low-affinity nutritional AHR ligand, respectively, by means of mRNA sequencing. AhR dependency of observed effects was proved using BMMs from *Ahr*-knockout (*Ahr*$^{-/-}$) mice. In total, more than 1,000 differentially expressed genes (DEGs) could be mapped, covering a plethora of AHR-modulated effects on basal cellular processes, i.e., transcription and translation, but also immune functions, i.e., antigen presentation, cytokine production, and phagocytosis. Among DEGs were genes that are already known to be regulated by AHR, i.e., *Irf1*, *Ido2*, and *Cd84*. However, we identified DEGs not yet described to be AHR-regulated in Mph so far, i.e., *Slpi*, *Il12rb1*, and *Il21r*. All six genes likely contribute to shifting the Mph phenotype from proinflammatory to anti-inflammatory. The majority of DEGs induced through BaP were not affected through I3C exposure, probably due to higher AHR affinity of BaP in comparison to I3C.

Mapping of known aryl hydrocarbon response element (AHRE) sequence motifs in identified DEGs revealed more than 200 genes not possessing any AHRE, and therefore being not eligible for canonical regulation. Bioinformatic approaches modeled a central role of type I and type II interferons in the regulation of those genes. Additionally, RT-qPCR and ELISA confirmed an AHR-dependent expressional induction and AHR-dependent secretion of IFN-γ in response to BaP exposure, suggesting a potentially novel auto- or paracrine activation pathway of Mph.

[P45] Modulation of AhR expression/activity in normal human bronchial epithelial cells during the benzo[a]pyrene-induced epithelial-to-mesenchymal transition

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The aryl hydrocarbon receptor (AhR) regulates cancer progression in several cancer types. In this study, a chronic (12-week) exposure to benzo[a]pyrene (BaP), which is both AhR-activating and genotoxic environmental contaminant, has been found to induce epithelial-to-mesenchymal transition (EMT) in normal human bronchial epithelial HBEC-12KT cells. Early, after 2-week exposure to BaP, morphological changes were observed that were possibly linked to CDKN1A/p21 and SERPINB2 induction. The upregulation of expression of mesenchymal markers and EMT-related transcription factors, as well as an increased cell migration, were first observed after 8-week exposure to BaP. Both groups of genes were strongly upregulated in transformed mesenchymal-like cells at the end of the treatment. Although exposure to non-genotoxic AhR ligand TCDD itself did not transform HBEC-12KT cells into mesenchymal-like cells, the AhR-regulated gene expression contributed to a stepwise BaP-induced EMT progression. The expression of AhR target genes (CYP1A1, CYP1B1, TIPARP, IL1B, SERPINE1 and SERPINB2) was significantly induced up to 8 weeks of BaP or TCDD exposure; however, it decreased in transformed mesenchymal-like cells. Unlike TCDD, BaP induced CDKN1A/p21 expression, which might be linked to a unique pattern of expression of Krüppel-like factor 6 (KLF6) that has been previously shown to act as a dimerization partner of the AhR. Here, BaP upregulated, while TCDD downregulated KLF6 expression, which might contribute to differential CDKN1A/p21 regulation by BaP. We also identified a significant induction of KYNU (kynureninase) in transformed mesenchymal-like cells, which might contribute to observed downregulation of AhR activity in fully transformed mesenchymal-like cells, via decreasing levels of endogenous AhR agonists. However, the exact mechanism(s) of downregulation of AhR signaling in transformed HBEC-12KT should be further investigated. In conclusion, CDKN1A/p21, pro-inflammatory responses and extracellular matrix remodeling genes were found to be upregulated during BaP-induced bronchial epithelial cell transformation. Their induction probably depends both on the AhR transcriptional activity and on the AhR-dependent metabolism of BaP that yields further active compounds.

[This study was supported by the Czech Science Foundation, project no. 24-10086S. The cell models were kindly supplied by Dr. Steen Mollerup, National Institute of Occupational Health, Oslo.]

[P46] Time-dependent disruption of TCDD-elicited differential gene expression following deletion of a dioxin response element (DRE) in the Pkm loci

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) reprograms liver metabolism by switching pyruvate kinase expression from isoform M1 (*Pkm1*) to M2 (*Pkm2*). TCDD induced Pkm2 expression is mediated by the the aryl hydrocarbon receptor (AhR) following binding to a dioxin response element located between exon 3 and 4 of the *Pkm* gene. To further investigate the significance of *Pkm* isoform switching in TCDD elicited hepatoxicity, we examined gene expression in primary hepatocytes freshly isolated from a *Pkm* DRE-deleted mouse model (DRE^{Pkm}) treated with 10 nM TCDD for 2, 4, 8, 12, 24, 48, 72, 96 and 120 hours. A total of 4965 and 6070 differentially expressed genes (DEGs; fold change >1.5, P1t < 0.8) were identified in wild-type (WT) and DRE^{Pkm} hepatocytes, respectively, with 1385 DEGs unique to WT, 2490 DEGs unique to DRE^{Pkm} and 3580 DEGs in common between both models. *Pkm* DRE deletion did not affect the expression of known direct AhR targets including *Cyp1a1*, *Cyp1a2*, and *Nqo1*. However, the expression of antioxidant defense related genes, including glutathione transporters, were diminished in DRE^{Pkm} mice. Additionally, clusters of genes exhibited delayed induction in DRE^{Pkm} hepatocytes resulting in more DEGs at 8 hours. Overall, these results suggest that PKM2 expression serves other important roles in response to TCDD beyond glycolytic reprograming in support of cell proliferation and antioxidant defenses. *This project is funded by R01ES029541 and the SRP P42ES004911.*

[P47] AhR-activating polycyclic aromatic hydrocarbons and disruption of glucose metabolism in hepatocyte-like cells

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Polycyclic aromatic hydrocarbons (PAHs) are widely distributed environmental pollutants that are present both in the diet and in the environment. For a long time, they have been primarily studied as genotoxic and carcinogenic toxicants; however, many PAHs are also potent AhR ligands that can interfere with signaling pathways controlling immune and endocrine signaling. Using a set of PAHs representing both weak and strong AhR ligands among PAHs, we studied their effects on metabolism, in particular metabolism of glucose and lipids in the cellular models derived from hepatocytes: differentiated hepatocyte-like HepaRG cells, immortalized MIHA hepatocytes and HepG2 hepatoblastoma cells. We found that similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, exposure to benzo[*k*]fluoranthene (BkF), which belongs among the PAHs that strongly activate AhR, significantly decreased glucose release into the glucose-free medium after 48h exposure in differentiated HepaRG cells. At the same time, BkF reduced mRNA levels of SLC2A2/GLUT2 (a major glucose transporter in liver cells) and SLC2A9/GLUT9 (which acts as a transporter of uric acid, glucose and fructose). Simultaneously, mRNA levels of phosphoenolpyruvate carboxykinase 1 (PCK1), rate-limiting enzyme in both gluconeogenesis and glyceroneogenesis, were strongly reduced upon BkF exposure. Downregulation of their expression occurred in a dose-dependent manner already at nanomolar concentrations, it started early upon BkF exposure, and it persisted in HepaRG cells. A similar effect was observed also in other liver cell models used and the functional role of AhR in these effects was validated using AhR knockout cell model. Collectively, these results suggest that exposure of liver cells to BkF and other potent AhR ligands among PAHs may, in a similar manner as exposure to persistent AhR ligands, impair their ability to *de novo* produce and transport glucose. This finding also prompts us to study more deeply of the relationship between PAHs (and their metabolites), AhR activation and impaired intracellular glucose metabolism and transport.

Funded by the Czech Science Foundation, project no. 24-10086S.

[P48] Sex specific alteration in microbiome composition and glycosylation patterns after long term TCDD treatment of C57BL/6 mice analyzed by flowcytometry

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Toxic compounds can affect the microbiota of the gut directly or via liver toxicity. Microbiota changes can be studied by 16S sequencing, or by multi-parameter flow cytometry using, e.g., scatter characteristics and DNA-dyes. According to literature reports, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a compound notorious for its (immune-)toxicity, changes the microbial pattern in the gut. However, high TCDD doses were used, which might cause liver toxicity, resulting in bile acids changes. We asked here, if TCDD affects the gut microbiome also at low doses in the absence of liver damage. Male and female C57BL/6 mice were orally administered 1µg/kg TCDD for 12 weeks (100 times less than the LD50 in mice). This dosing was harmless for the gut and only had minimal impact on the liver. Next, we assessed the microbial patterns in fecal pellets and cecal samples of TCDD-exposed mice. 16S sequencing revealed no differences. Surprisingly however, analysis by flow cytometry identified significant differences in the microbiota community profile upon TCDD exposure in female mice. While 16S identifies microbes by the genome, flow cytometry has the advantage that it might pick up in addition phenotypic changes such as glycosylation or a tendency of bacteria to stick together. We therefore stained the bacteria with a panel of fluorescent sugar-specific lectins. Indeed, this unveiled significant alterations in the glycosylation patterns of TCDD-treated mice versus controls. Again, this was more pronounced in female than in male mice. Dysregulation of glycosylation patterns by toxic compounds is a novel finding, which has implications for the health of the host because glycoconjugates govern biofilm forming, infectious behavior of bacteria, and host immune responses. We posit that changes in glycosylation and bacterial community structure are relevant parameters in assessing the toxicity of chemical compounds in the gut in male and female mice.

[P49] Thermoneutral housing attenuates 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) hepatotoxicity in male C57BL/6 mice

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Studies suggest housing mice at thermoneutral (T_N^M; 28 – 30° C) instead of thermostandard (T_S; 20 – 24° C) ambient temperatures better reflects homeostatic ambient conditions. Thermoneutral conditions minimize cold stress in mice, thereby providing an environment that more closely reflect human physiological conditions. At T_S, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) dose-dependently induced AHR-mediated non-alcoholic fatty liver (NALFD)-like pathologies including reversible hepatic steatosis that can progress to steatohepatitis with periportal fibrosis and biliary hyperplasia in male C57BL/6 mice depending on the dose. To investigate the effect of housing temperature on TCDD-induced hepatotoxicity, male C57BL/6 mice were orally gavaged every 4 days for 28 days with 0.03 – 30 µg/kg TCDD or sesame oil vehicle under T_S and T_N^M conditions. Mice at T_N^M exhibited less relative liver weight gain and more modest steatosis with less severe triglyceride accumulation, lipid peroxidation, immune cell infiltration, and hepatocellular hypertrophy. T_S mice exhibited greater serum ALT increases and reductions in serum glucose and triglyceride levels at 30 µg/kg TCDD. Bulk liver RNA-seq analysis identified fewer differentially expressed genes (DEGs) at T_N^M, although the overlap between T_N^M to T_S DEGs was comprable. Contrary to hepatotoxicity assessments, benchmark dose analysis of DEGs revealed AHR-battery gene induction was more sensitive at T_N^M (*e.g.*, *Cyp1a1*). In addition, comparable AHR-battery gene induction in liver and epididymal white adipose tissues, suggested the attenuated hepatotoxicity at T_N^M was not due to reduced hepatic TCDD accumulation. Increased serum FGF21 and altered serum and hepatic thyroid hormones at T_S compared to T_N^M suggested systemic and liver-associated disruption of thermoregulation was more severe in TCDD-exposed mice at T_S. Overall, the results demonstrate TCDD hepatotoxicity was reduced at T_N^M, not due to differences in toxicokinetics, AHR-battery expression, or in the mechanism by which TCDD induces hepatotoxicity.

WJS was supported by T32ES007255. This project was funded by R01ES029541.

[P50] Aryl Hydrocarbon Receptor shapes transcriptional responses to Interleukine-4 by regulating STAT6 binding modalities

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Immune cells can rapidly adapt their functional program in response to cytokines. How cytokine-induced transcriptional responses are affected by micro-environmental cues remains poorly understood. The Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor sensing environmental signals including metabolites. Here we addressed the crosstalk between Interleukine-4 (IL-4) and AhR signaling in monocytes. We show that AhR controls a module of the IL-4 response program and regulates the IL-4-induced metabolic adaptation of monocytes. Mechanistically, we found that AhR activation shapes STAT6 binding pattern by controlling the kinetics of STAT6 phosphorylation. Our results identify AhR as a key player in the molecular control of cytokine responses and reveal the profound impact of the cellular context on the functional responses to IL-4.

[P51] Exploring the Role of Aryl Hydrocarbon Receptor (AHR) in High-Fat Diet-Induced Obesity

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The Aryl Hydrocarbon Receptor (AHR) is a key regulator of inflammation, metabolic homeostasis, and lipid and glucose metabolism. This suggests that AHR may play a critical role in chronic lifestyle diseases such as diet-induced obesity and fatty liver disease. To test this, we generated *Ahr* knockout (*Ahr*$^{-/-}$) mice by crossing *Ahr*^{fx} (*Ahr*$^{tm3.1Bra}$/J) mice with *CMV-Cre* (B6.C-Tg(CMV-cre)1Cgn/J) mice. Wild-type (WT) mice and *Ahr*$^{-/-}$ mice were then exposed to a 60% high-fat diet for 5 weeks. Body weight, body composition, fecal and blood samples were collected weekly. At the end of the experiment, fasted oral glucose tolerance tests were performed for both diet groups. Liver, white adipose tissue (WAT), brown adipose tissue (BAT), spleen and colons were also harvested for gene expression analyses and for histopathological assessment. *Ahr*$^{-/-}$ mice exhibited reduced body weight gain which included reduced increase in fat mass coupled with less severe loss of lean body mass compared with their WT counterparts. *Ahr*$^{-/-}$ mice on control diet displayed improved blood glucose tolerance compared with WT, which was lost in *Ahr*$^{-/-}$ mice on a high-fat diet. Consistent with other studies, *Ahr*$^{-/-}$ mice had reduced liver weights and increased spleen weights in both control and high-fat diet fed groups compared with WT. The inflammatory marker *Il-6* was increased in the WAT of WT mice on a high-fat diet, but not in the *Ahr*$^{-/-}$ mice. Interestingly, *PPAR-γ*, a pivotal regulator of adipogenesis, was increased in *Ahr*$^{-/-}$ mice in both WAT and BAT, despite these animals having reduced body fat. Reduced levels of the pro-inflammatory gene, lipocalin 2, were also observed in colon tissue from *Ahr*$^{-/-}$ mice on high-fat diet. Our findings suggest a role for AHR in modulating fat metabolism, as evidenced by reduced weight gain and altered tissue gene expression responses. However, additional studies are needed to fully characterize the mechanisms by which AHR contributes to these observed effects.

[P52] Aryl hydrocarbon receptor ligands increase ABC transporter activity and expression in killifish (Fundulus heteroclitus) renal proximal tubules

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Many widespread and persistent environmental organic pollutants, for example, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and some polychlorinated biphenyls, activate the aryl hydrocarbon receptor (AhR) causing it to translocate to the cell nucleus where it transactivates target genes, increasing expression of a number of xenobiotic metabolizing enzymes as well as some transporters. No so much is known about the ability of AhR to target transporters within the kidney. We show here that exposing isolated and functionally intact killifish (Fundulus heteroclitus) renal proximal tubules to micromolar concentrations of β-naphthoflavone (BNF) or nanomolar concentrations TCDD roughly doubled the transport activity of the ABC export proteins multidrug-resistance-associated-proteins Mrp2 and Mrp4, P-glycoprotein (P-gp) and Breast cancer resistance protein (Bcrp). All these ATP-driven xenobiotic efflux pumps are critical determinants of renal xenobiotic excretion. These effects were abolished by actinomycin D and cycloheximide and by the AhR antagonist, α-naphthoflavone, indicating that increased transport activity was dependent on transcription and translation as well as ligand binding to AhR. Quantitative immunostaining of renal tubules exposed to BNF and TCDD showed increased luminal membrane expression all 4 transporters under investigation, Mrp2, Mrp4, P-gp and Bcrp. Thus, in these renal tubules, the 4 ABC export proteins are targets of AhR action.

[P53] Bariatric Surgery Reduces Intestinal AhR Signalling and Increases Colitis Severity in Mice

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Obesity increases the risk of a multitude of metabolic and inflammatory diseases including increased susceptibility to inflammatory bowel disease (IBD). Bariatric surgeries remain the most effective strategy for effecting short- and long-term weight loss. Despite the evidence that bariatric surgery appears safe for patients with existing IBD, several clinical studies have revealed a link with the *de novo* development of IBD after the surgery. Consistent with these clinical studies, we found bariatric surgery increases DSS-induced colitis severity. There is some clinical evidence that bariatric surgery alters indole metabolism; however, interpretation of these data are confounded because both weight loss and surgery can impact microbial indole metabolism. We, therefore, developed and validated a mouse model of bariatric surgery to deconvolute weight-loss-dependent versus surgery-dependent effects on indole metabolites. Roux-en-Y gastric bypass surgery led to a reduction in indole-3-propionic acid and kynurenic acid in a weight loss-independent manner. Consistent with this, intestinal expression of the AhR targe gene Cyp1a1was reduced by 90% in mice that underwent surgery compared to weight-matched, sham-operated control mice. We have also shown that supplementing mice with the synbiotic combination of *Bifidobacterium pseudocatenulatum* (probiotic) and the human milk oligosaccharide 2‘-fucosyl lactose (2‘-FL, prebiotic) reduces colitis severity in a DSS model of colitis. Supplementation with this synbiotic combination produces the AhR ligand indole-3-lactic acid, increases AhR activity and attenuates intestinal inflammation. Current work aims to determine if supplementation with AhR ligand-producing synbiotics can attenuate colitis risk after bariatric surgery.

[P54] Components of AGE-rich nutritional extracts exert complex cellular effects through simultaneous modulation of AhR, NRF2 and NF-kappaB

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In current literature, nutritional extracts rich in advanced glycation endproducts (AGEs) are described to activate inflammatory and anti-inflammatory signaling cascades or even exert organ-protective functions in *in vitro* studies. Those diverse processes are often attributed to the contained AGEs and the interaction with their receptor RAGE. However, the bioactive components in foods and extracts are usually described insufficiently, and secondary plant metabolites and roasting products can influence bioactivity. Furthermore, eliminating RAGE in cellular systems does not interrupt the responses completely. Thus, we hypothesized that an additional receptor with a broader ligand repertoire might be involved and that AhR might be the novel factor facilitating cellular responses of AGE-rich extracts.

To prove our hypothesis, we utilize HepG2-reporter cell lines expressing Luciferase upon activation of the transcription factors AhR, NRF2 or NF-kappaB to screen bread crust extract (BCE), known compounds of AGE-rich extracts as well as other putative components for their activation potentials and validate target-gene expression in non-reporter cells. Furthermore, we investigate the CYP1A1 activity of stimulated cells by an ethoxy resorufin-O-demethylase (EROD) assay.

We could demonstrate differential activation of the AhR, NRF2 and NF-kappaB reporters by BCE and different components that are known AhR-agonists like kynurenine and benzo[a]pyrene. Furthermore, we could exclude several free AGEs, like carboxymethyl-lysine (CML) and Methylglyoxal-hydroimidazolone (MG-H1), as bioactive components. However, one AGE structure showed differential activation of AhR and NRF2 reporters, and a binding to the AhR binding pocket could be successfully modeled. Target gene transcription and CYP1A1 activation were also validated in non-reporter HepG2 cells for selected compounds.

Thus, we conclude that known and novel AhR ligands can be found in AGE-rich extracts like BCE and that their interplay influences the overall cellular response by modulating AhR, NRF2 and NF-kappaB transcription factor activation.

[P55] Investigating the effect of transient vs sustained AhR activation on zebrafish neuromasts regeneration

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Neuromasts are zebrafish mechanosensory organs that are crucial for sensing and responding to dynamic changes in the surrounding water. Different from mammalian sensory cells, the cells of the neuromasts can undergo a complete regeneration after ablation. The process of regeneration depends on a functioning innate immune response, followed by proliferation and differentiation of neuromast resident progenitor stem cells. As the aryl hydrocarbon receptor (AhR) is known to be involved in cell proliferation, stemness maintenance and differentiation, we asked whether ligands initiating different duration of activation may interfere with the well-established neuromasts regeneration following copper sulphate mediated ablation.

For this, we selected 3,3',4,4',5-Pentachlorobiphenyl (PCB126) as a xenobiotic ligand inducing a sustained AhR activation, while 3-Methylcholanthrene (3-MC) and Indolo[3,2-b]carbazole (ICZ) were chosen as a xenobiotic and natural ligand, respectively, inducing a transient activation. We found that upon injury, the AhR activators exert distinct effects on the macrophage recruitment, where 3-MC caused a reduced recruitment, while PCB126 caused a retention of the macrophages at the damage site, and ICZ showed no effect. Furthermore, the compounds affected the neuromast cell proliferation, with 3-MC causing a pronounced inhibitory effect, while both PCB126 and ICZ appeared to enhance it.

Keywords: zebrafish neuromasts, copper sulphate ablation, regeneration, macrophage recruitment

[P56] The modern human aryl hydrocarbon receptor is less active than the Neandertal version

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor involved in a variety of physiological functions, including the response to toxicants. The main target genes of AHR are cytochrome P450 genes, which encode enzymes that metabolize aromatic hydrocarbons. AHR in present-day humans differs from the AHR of Neandertals, Denisovans and other primates in that it has an alanine to valine substitution at position 381. Previous studies where the ancestral and modern versions of AHR were overexpressed in cell lines have yielded contradictory results. Here, we introduced the codon for the ancestral, i.e. Neandertal-like amino acid into human cells using CRISPR/Cas genome editing and measured the expression of target genes with RNA-Seq and RT-qPCR. We show that cells expressing the ancestral AHR are similar to chimpanzee cells in that they express higher levels of AHR target genes in the absence of exogenous ligands than cells expressing the modern AHR. Furthermore, higher doses of the endogenous tryptophan metabolite kynurenic acid and the environmental pollutant benzo(a)pyrene are required to induce the expression of AHR target genes in cells expressing the modern AHR than in cells expressing the ancestral AHR. Thus, the modern human AHR affects the expression of many of its target genes to a lesser extent than the ancestral variant seen in archaic hominins.

[P57] New insights into AHR activation by Trp-derived metabolites

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The catabolism of the essential amino acid tryptophan (Trp) by its main degrading enzymes indoleamine-2,3-dioxygenase 1 and 2 (IDO1/2), tryptophan-2,3-dioxygenase (TDO2) as well as interleukin-4-induced gene 1 (IL4I1) is known to play an important role in cancer progression. While IDO1/2 and TDO2 catalyze the conversion of Trp to kynurenine (Kyn), the first step of the Kyn pathway, the L-amino acid oxidase IL4I1 degrades aromatic amino acids to their respective alpha-keto acids thereby producing hydrogen peroxide and ammonium. Trp-derived metabolites lead to the activation of the transcription factor aryl hydrocarbon receptor (AHR) thereby promoting tumor cell motility and immunosuppressive functions. As treatments targeting Trp degradation are gaining in importance, efficient and precise measurements of Trp levels are highly relevant. We developed a multiplex approach using tandem mass spectrometry combined with tandem mass tags for measuring Trp and its downstream metabolites that enables simultaneous quantitative comparison of Trp and its metabolites in multiple samples. Using this technology, we have characterized Trp catabolism in cell culture, mouse models and human samples. Our metabolomics analyses reveal new insights into the role of Trp-derived metabolites and their impact on AHR activation.

[P58] Exploring the AhR molecular pathways to improve gut microbiota and liver injury in alcohol-associated liver disease.

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Alcohol-associated liver disease (ALD) is the leading cause of cirrhosis worldwide, with few therapeutic options other than alcohol withdrawal. Intestinal microbiota (IM) plays a causal role in the severity of the disease both in mice and humans. Among metabolites produced by IM, bile acids and tryptophan metabolites are mainly modified in ALD. We previously demonstrated that pectin, a soluble fiber, improves liver and gut injuries in ALD through bile acid metabolism and indoles production acting through the aryl hydrocarbon receptor (AhR) signaling. However, the potential of tryptophan metabolites to improve ALD through the AhR and which cells are involved in mechanisms remain unknown. In this study, we aimed to decipher the involvement of AhR, specifically in the liver and the gut, by establishing conditional knockout mice models for AhR in hepatocytes, enterocytes, or dendritic cells (DCs) and CD11c expressing macrophages, followed by implementing preventative treatments with tryptophan or pectin in the context of ALD. Based on metagenomic and metabolomic analyses, we focused on changes in IM and microbiota tryptophan metabolites, which are ligands of AhR. We found that a tryptophan-enriched diet partially reproduced the effect of pectin without major changes in IM but with significant modification in the relative abundance of some bacteria. The tryptophan diet also modifies tryptophan metabolism and AhR ligands. These changes are closely related to IM but differ from the effect of the pectin diet. Serum tryptophan levels increased in the tryptophan group but not in the pectin group, while the serum tryptamine levels exhibited the opposite trend. Through conditional AhR knockout mice, we observed that AhR deficiency in hepatocytes or enterocyte-expressing cells worsened the ALD. However, the protective effect of pectin and tryptophan is not abrogated by AhR deficiency. In these AhR deficient mice, pectin and tryptophan treatments improve the alcohol-induced liver injury, conversely, the improvement of the gut barrier was abrogated. Overall, our data showed that tryptophan reproduces a part of the effect of pectin on the improvement of liver and gut injuries, which is independent of the AhR expression in hepatocytes and enterocytes. Conversely, the improvement of the gut injury depends at least on part of the AhR expression in the gut but not in the liver, suggesting that gut improvement is not a prerequisite to improve the liver injury.

[P59] Minibioreactor arrays (MBRAs) to model microbiome response to tryptophan and alcohol in the context of alcohol-associated liver disease (ALD)

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Intestinal microbiota (IM) plays a causal role in the severity of alcohol-associated liver disease (ALD). Using IM transplantation in mice, we proved that the dysbiosis of alcohol use disorder (AUD) patients with severe alcohol-associated hepatitis (sAH) could be modified, leading to an improvement in alcohol-induced liver injury by increasing tryptophan metabolites to activate aryl hydrocarbon receptor (AhR) signaling pathway. However, the effect of tryptophan on IM in AUD patients, as well as its interactions with alcohol, remain to be elucidated. For this purpose, we used an in vitro approach with Minibioreactor arrays (MBRAs) that allows for the study of IM in a continuous-flow culture with well-controlled factors. Fecal samples from AUD patients with sAH (n=2) or with noAH (n=2) were transferred to MBRAs chambers. After 24 hours of adaptation in the initial medium, treatments with different tryptophan concentrations (low: 8mg/L, normal: 24mg/L and high: 72mg/L) were initiated for 48 hours. Subsequently, alcohol was introduced in the system for 5 days (50mM ethanol/Day). Finally, alcohol was removed and the cultures were maintained for an additional 5 days. IM analysis was conducted by 16s sequencing. AhR activity of tryptophan derivatives in supernatants was determined using two reporter lines: intestinal epithelial cells (HT-29) and hepatocytes (HepG2) labelled with Lucia-AhR. After 24h of stabilization, MBRA effectively maintains each fecal community. Tryptophan had no effect on the alpha and beta diversity of the IM from sAH and noAH patients. However, normal tryptophan level decreased the relative abundances of *Escherichia – Shigella* and increased *Bacteroides* in noAH IM, decreased *Proteobacteria* and increased *Bacillus* in sAH IM. In the absence of alcohol, tryptophan changed more number of bacteria in noAH IM (43 species) than in sAH IM (8 species). However, with alcohol conditions, tryptophan had minimal effect on the noAH IM. Compared to low tryptophan, normal and high tryptophan levels increased the AhR activity. Overall, our results suggest that maintaining a normal tryptophan level in patients with noAH could be essential to prevent dysbiosis and high concentrations of tryptophan may have a beneficial effect on the IM of sAH patients. Tryptophan holds potential as a novel therapeutic agent for ALD treatment but these results must be confirmed in vivo.

[P60] Acute physical exercise activates AHR target genes in PBMCs

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Exercise reroutes the kynurenine pathway (KP) of tryptophan metabolism towards kynurenic acid (KA) in skeletal muscle and systemically, thereby increasing systemic levels of potent endogenous ligands for the aryl hydrocarbon receptor (AHR). We investigated whether different acute aerobic exercise modes increase the expression of AHR target genes in PBMCs. Blood samples from 24 healthy adults (age: 29.7 ± 4.3 years; women: 12) were collected immediately before, after, and 1h after an acute exercise session of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), respectively. The order of both sessions was randomized and a wash-out period of seven days was implemented. Both sessions were matched for workload and duration (50 min). The HIIT session consisted of six 3-min bouts at 90% V̇O2peak with active breaks. The MICT session consisted of 50 min continuous running at 70% V̇O2peak. Expression of AHR target genes was assessed using qRT-PCR. Additionally, levels of kynurenine and KA were measured in blood serum using HPLC MS/MS to examine greater AHR ligand availability in response to exercise. Analyses of variance with repeated measures and Bonferroni-corrected post hoc tests were conducted to evaluate statistical differences within and between both exercise modes. Increased systemic levels of kynurenine and KA were observed in response to both exercise modes ($p<.01$). Both exercise modes significantly increased the expression of the *AHR* target genes *AHR*, *AHRR*, and *CYP1B1* ($p<.05$) up to 5-fold (*AHRR*), but effects of HIIT were statistically greater compared to MICT. In conclusion, acute aerobic exercise increases systemic levels of the endogenous AHR ligands kynurenine and KA, and activates AHR target genes in PBMCs in an exercise intensity-dependent manner. These data indicate an acute exercise-induced AHR activation in circulating immune cells, representing a potential novel mechanistic link to longer-term anti-inflammatory effects of exercise, and are possibly of relevance for various chronic diseases.

[P61] Development of novel approaches for the detection of AHR activity

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that enables cells to adapt to environmental, nutritional or metabolic changes. Upon activation by exogenous or endogenous ligands the AHR translocates to the nucleus to form a heterodimer with the AHR nuclear translocator (ARNT). In complex with ARNT, the AHR binds to xenobiotic response elements and initiates transcription of its target genes with broad roles in development, immunity, and cancer. However, expression of these target genes varies greatly between different cell types and in response to different AHR ligands, which points towards more complex mechanisms of AHR activation. Analysis of single target genes is therefore not sufficient to predict AHR activity in response to a given stimulus. Hence, complementary approaches to reliably measure AHR activation are needed. We are currently developing novel protein-based methods to detect AHR activity in response to different AHR ligands in different disease contexts. We are validating our findings by a broad ensemble of classical AHR activity readouts. Our new tools for the measurement of AHR activity will contribute to a better understanding of AHR activity in different disease contexts and may help identifying potential novel AHR ligands as well as AHR interaction partners.

[P62] Endothelial function is modulated by AGE-rich nutritional extracts through the Aryl-hydrocarbon receptor

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With innovation in modern health care, we see an increase in human lifespan. However, aging-associated diseases, including cardiovascular pathologies, still limit healthy aging. Advanced glycation end-products (AGEs) have come into focus as contributors to these complex pathologies. AGEs are endogenously generated in a non-enzymatic process or consumed through food as dietary AGEs (dAGEs). While the accumulation of endogenous AGEs often increases oxidative stress and causes inflammaging, the role of nutritionally-derived AGEs is less clear. The detection and detoxification of AGEs is facilitated by multiple receptors, of which RAGE (the receptor for AGEs) is widely known. However, previous results imply the involvement of additional signaling mediators besides RAGE.

Here, we establish the aryl hydrocarbon receptor (AhR) as a novel receptor for compounds of AGE-rich extracts like bread crust extract (BCE), one being the known AhR-ligand kynurenine.

Utilizing stimulation with BCE or single AGEs, we show AhR nuclear translocation through immunofluorescence staining while also validating target gene induction in the endothelial cell line EA.hy926. To prove the induction of enzymatic activity of the AhR-dependent CYP1A1 enzyme, we employed the EROD assay. Furthermore, we measure AhR protein abundance over a period of 24 hours to explore subsequent degradation via the 26S proteasome.

Our findings prove nuclear translocation of AhR in response to different compounds of AGE-rich extracts, where AhR induces CYP1A1 gene expression up to threefold within 24 hours. This is consistent with an increase of enzymatic activity, which decreased with AhR inhibitor treatment. After successful induction of target genes, we see degradation of AhR protein. The kinetic of degradation varies depending on both the ligand as well as the cell line. Generally, BCE shows a decelerated kinetic compared to single AGE treatments. Furthermore, we see an induction of NRF2 and NFκB signaling along with AhR stimulation in EA.hy926 cells, highlighting the crosstalk between these signaling pathways.

Ultimately, we aim to affirm our findings and further differentiate between AhR and RAGE-mediated signaling by generating CRISPR/Cas9 knockout clones in endothelial cells. Once established, we will investigate the impact of the knockout on cellular function. Based on our findings, we conclude a function for AhR as a mediator of AGE-induced signaling.

[P63] Epigenetic Modifications Control CYP1A1 Inducibility in Human and Rat Keratinocytes

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Serially passaged rat keratinocytes exhibit dramatically attenuated induction of *Cyp1a1* by aryl hydrocarbon receptor ligands such as TCDD. However, the sensitivity to induction can be restored by protein synthesis inhibition. Since previous work revealed that the functionality of the receptor was not affected by passage, the present work explored the possibility of epigenetic silencing on CYP1A1 inducibility in both rat and human cells. Use of an array of small molecule epigenetic modulators demonstrated that inhibition of histone deacetylases mimicked the effect of protein synthesis inhibition. Consistent with this finding, cycloheximide treatment also reduced histone deacetylase activity. More importantly, when compared to human *CYP1A1*, rat *Cyp1a1* exhibited much greater sensitivity toward epigenetic modulators, particularly inhibitors of histone deacetylases. Other genes in the aryl hydrocarbon receptor domain showed variable and less dramatic responses to histone deacetylase inhibitors. These findings highlight a potential species difference in epigenetics that must be considered when extrapolating results from rodent models to humans and has implications for xenobiotic- or drug-drug interactions where CYP1A1 activity plays an important role.

[P64] Exploring the role of the AhR/AhRR signaling pathway in adipose tissue organoids

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High calorie intake combined with reduced physical activity leads to adipose tissue dysfunctionality and obesity and thus increases the risk for comorbidities such as type 2 diabetes and heart disease. The AhR plays an essential role in obesity, since a lack of AhR signaling by genetic knockout or pharmacological inhibition protects from diet-induced weight gain in mice. The AhR repressor (AhRR) is expressed upon AhR signaling and regulates the pathway in a cell type-specific manner. Interestingly, our in vivo data reveal that AhRR-deficient mice are protected from high fat diet-induced obesity and metaflammation, similar to AhR-deficient mice.

To examine the role of the AhR/AhRR in adipose tissue more closely, we developed a macrophage containing 3D adipose tissue organoid model, which allows us to analyze the interplay between different cell types in adipose tissue in a more physiological setting. For this, the stromal vascular fraction (SVF) of different fat depots was isolated, expanded in 2D, followed by the formation and differentiation of adipocytes to a 3D organoid in a scaffold-free environment. To modulate AhR signaling in these organoids, the cultures were supplemented with AhR ligands and lipids, and organoids that were deficient for different AhR signaling components were analysed.

Using AhRR reporter mice, we observed that the AhRR was expressed by macrophages. AhRR-deficiency did not influence macrophage frequencies in organoids but led to a modest reduction of the organoid size and a decrease in differentiation markers. In line with the size reduction, mass spectrometry-based lipidomic analysis revealed that AhRR-deficiency decreased the overall lipid content. In order to analyse the cellular crosstalk in more detail, cell type-specific AhRR-deficient organoids were generated and are currently investigated.

Overall, we conclude that AhR signaling plays an important role in the macrophage-adipocyte interaction in adipose tissue organoids.

[P65] Aryl hydrocarbon receptor is involved in lifespan promotion by pomegranate extract in *Caenorhabditis elegans*

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Pomegranate extract has many beneficial properties, such as increasing lifespan, but nothing is known about the mechanism of action involved or whether it affects vitality. Our study proposes to elucidate whether the mechanism of action that increases lifespan is through aryl hydrocarbon receptor pathway and to study the effect of pomegranate extract on healthspan determined as maintenance of motility in *C. elegans*. This finding could be very important to improve the quality of life in aging. We have performed lifespan studies in *C. elegans* supplemented with pomegranate extract and we have evaluated the motility with assessment vitality studies. Furthermore, we have studied the effect of pomegranate extract in worms of two different aryl hydrocarbon receptor mutants. Our results show that aryl hydrocarbon receptor is involved in the increase in life- and healthspan in worms supplemented with pomegranate extract.

[P66] AhR deficiency in mice causes dysregulation of stress erythropoiesis and enhanced susceptibility to *Salmonella* Typhimurium infection

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The Aryl hydrocarbon Receptor (AhR) is a ligand-activated transcription factor, acting as an environmental sensor for pollutants, diet-derived ligands or bacterial metabolites. Besides its function in xenobiotic metabolism, the AhR regulates acute innate as well as adaptive immune responses against pathogens.

Infection with *Salmonella enterica* serovar Typhimurium (STM) in mice leads to a lethal systemic disease that resembles human typhoid fever caused by *S. enterica* serovar Typhi. By using the attenuated STM strain TAS2010, we investigated the impact of AhR signaling in a mouse model for chronic systemic STM infection. AhR-deficient ($AhR^{-/-}$) mice were highly susceptible to TAS2010 infection compared to WT mice as indicated by reduced bacterial clearance in liver and spleen and increased mortality. Furthermore, STM infection led to macrocytic anemia in $AhR^{-/-}$ mice caused by a shutdown in bone marrow erythropoiesis. As a consequence, erythropoiesis was shifted into the spleen, which resulted in a vast expansion of immature erythroid cells, mainly accounting for enhanced splenomegaly in $AhR^{-/-}$ mice. In addition, $AhR^{-/-}$ mice showed a massive destruction of the splenic microarchitecture, especially in T and B cell follicles as well as the marginal zone. Elevated serum levels of erythropoietin and interleukin-6 upon infection as well as increased numbers of splenic stress erythroid progenitors already in steady state probably drive the enhanced extramedullary erythropoiesis in $AhR^{-/-}$ compared to WT mice. Thus, this increased infection-induced stress erythropoiesis might be causative for the alterations in splenic immune cell compartments, thereby disturbing an effective host defense of $AhR^{-/-}$ mice against chronic STM infection.

[P67] Computational Perspectives on Aryl Hydrocarbon Receptor Signalling Pathway

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The Aryl Hydrocarbon Receptor (AhR) plays a pivotal role in cellular responses to environmental stimuli, by employing a multi-step transformation mechanism going from ligand binding to DNA interactions. Despite limited experimental structures have been available over two decades, advancements in structural insights were achieved through homology modelling based on bHLH-PAS protein systems as templates. In this presentation we review our recent contributions to the field.

Our computational modelling allowed us to decipher the binding modes of a group of chemicals, representative of major AhR ligand classes, taking a significant stride toward the understanding of ligand effects in the AhR activation mechanisms[1]. Notably, molecular dynamics simulations allowed us to investigate the impact of TCDD on the AhR:ARNT complex stability, unravelling mechanisms by which ligand-induced perturbations propagate to the DNA recognition site, pivotal for gene transcription[2]. In parallel, our investigations on the AhR:hsp90 cytosolic complex, based both on the Cryo-EM structure of hsp90 bound to the client CDK4 protein and on experimental evidences, suggested a binding mode where the AhR threads through the two hsp90 subunits, leaving the PAS-A and PAS-B repeats on the opposite sides of hsp90, with the bHLH bound to the N-terminal domain of the chaperone[3]. This innovative arrangement found confirmation in recent Cryo-EM structures of hsp90 bound to AhR[4,5], thus marking a structural breakthrough that opens avenues for computationally assessing the effects of ligands on the cytosolic complex.

In this context, we underscore the need for an integrated approach, combining molecular computational studies with experimental research. This interdisciplinary strategy would provide invaluable insights into the AhR signalling pathways[6]. These findings also hold great potential for therapeutic applications by unravelling the molecular features of ligands that trigger specific AhR biological responses.

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[P68] Circadian tryptophan metabolites confer a daily rhythm of AHR activity.

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Notwithstanding acute diet-phytochemical derived activation, host and microbial tryptophan metabolism represent the dominant route to endogenously mediated stimulation of physiological Ah receptor (AHR) activity. Previous rodent studies have revealed circadian fluctuations in both tryptophan metabolism and AHR activity. Such studies have been conducted using standard plant based rodent chow, known to contain complex mixtures of phytochemical AHR modulators, and thus establish a logical nocturnal circadian AHR ligand consumption-AHR activation rhythm. It is unclear however, whether tryptophan metabolism provides a, phytochemical independent, circadian AHR tone. Using female C57BL6/J mice maintained on a nocturnally restricted feeding schedule (i.e. dark-phase, ad libitum access) with a nutritionally defined diet devoid of phytochemicals (AIN93G), we have examined the contribution of tryptophan metabolism towards circadian AHR activity. Analysis of peripheral circadian indicators (*Bmal1*, *Nr1d1*) establish that a robust biological clock is maintained in this model. Time-resolved, targeted LCMS metabolomic, gene and protein expression analyses reveal circadian cycling of hepatic tryptophan metabolizing enzymes (*Tdo2, Tat, Got1, Got2, Kat1, Kat2 and Il4i1*) and serum tryptophan metabolites (indole-3-acetate, indole-3-lactate, indole-3-propionate, indole aldehyde, kynurenine, kynurenic acid) previously established as AHR ligands. Concordantly, we observed cyclical hepatic AHR activity directed by circadian feeding. Overexpression studies indicate that tryptophan transaminase activity derived from TAT, GOT1/2 and IL4I1 is sufficient to generate indole-3-pyruvate derived AHR ligands. These data suggest that a circadian rhythm of tryptophan metabolism orchestrates a daily tone in AHR activity that likely modulates AHR dependent physiology. Furthermore, the enhanced sensitivity of human AHR towards these endogenous tryptophan metabolites, relative to rodent AHR suggests circadian AHR physiology is accentuated and thus more sensitive to circadian stress.

[P69] Analysis of the regulation of tryptophan-degrading enzymes in AHR activation

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In addition to its many other functions, the ligand-activated transcription factor aryl hydrocarbon receptor (AHR) is a key regulator of tumor progression. Many tumors express high levels of the AHR and produce endogenous AHR agonists, thus taking advantage of AHR activation to promote tumor cell intrinsic malignant properties and to suppress anti-tumor immune responses. Derivatives of the essential amino acid tryptophan represent an important class of endogenous AHR ligands. The kynurenine pathway, initiated by indoleamine-2,3-dioxygenase 1/2 (IDO1/2) or tryptophan-2,3-dioxgenase (TDO2), is considered as the main tryptophan-catabolic route in humans, yielding the AHR agonists kynurenine and kynurenic acid. Cancers express high levels of IDO1 or TDO2, taking advantage of tryptophan catabolite-mediated AHR activation. Inhibition of IDO1 or TDO2 thus represents a promising strategy to target both cancer cell malignancy and tumor-derived immunosuppression and to potentially overcome therapy resistance in patients with hyperactive AHR. However, AHR activation is more complex, as the AHR cross talks with many other transcription factors and signaling pathways, which directly affect the AHR in a tissue- and context-specific manner. Also, expression and activity of IDO1 and TDO2 depend on other transcription factors and signaling pathways that may indirectly contribute to the context-specificity of AHR activation. Hence, a better understanding of the crosstalk of IDO1 and TDO2 with other transcription factors and signaling pathways may help in stratifying patients for therapies targeting the AHR.

[P70] A critical role of CYP1 Enzymes in regulating AHR Signalling and Metabolism

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The adverse effects at exposure to xenobiotic aryl hydrocarbon receptor (AHR) ligands are generally ascribed to their slow clearance or to formation of toxic ligand metabolites, often mediated by the AHR-regulated cytochrome P4501 (CYP1) enzymes. Yet, several studies have demonstrated a critical role for CYP1 enzymes in maintaining a homeostatic AHR signalling by catalysing the biotransformation of natural AHR ligands, thereby curtailing their duration of activation. This concept is further underpinned by our studies where constitutive expression of CYP1A1 led to defunct AHR signalling, while deletion of CYP1 enzymes potentiated AHR activation as a result of reduced ligand clearance.
AHR signalling is known to impact metabolic pathways. However, the role of CYP1-mediated feedback regulation on metabolic functions of AHR is largely unknown. In this study, we performed comprehensive serum metabolite profiling in mice with dysregulated AHR pathway in the absence of ligand exposure. Serum samples from male and female mice depleted of AHR (AHR-KO) or CYP1 enzymes (Cyp1a1-, 1a2-, and 1b1 triple KO), and mice with constitutive Cyp1a1 expression were analysed by targeted and untargeted GC/LC-MS. Using this approach, we identified several metabolic pathways significantly affected by altered AHR or CYP1 function, revealing a critical contribution of homeostatic AHR activity in the regulation of diverse metabolic pathways. Importantly, a strong correlation between metabolites altered upon AHR depletion and constitutive Cyp1a1 expression was observed, confirming a central role of CYP1A1 as negative regulator of metabolic AHR functions.

[P71] The EGF receptor determines ligand-specific AHR responses and serves as a cell-surface receptor for a variety of environmental organic pollutants.

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Understanding the molecular mechanisms and parameters that shape the ligand-specific outcome of the AHR response is the key to utilize the full therapeutic potential of AHR modulators. In this context, the complexity of non-canonical AHR signaling pathways comes into focus. In fact, AHR activation may affect a variety of cellular signaling pathways, including the epidermal growth factor receptor (EGFR) and downstream signal transduction, in a ligand-specific manner.

We have previously reported that AHR activation and the subsequent dissociation of the cytosolic AHR multiprotein complex by polycyclic aromatic hydrocarbons (PAHs) results in a biphasic stimulation of EGFR, i.e. an early c-Src-driven endogenous phosphorylation of EGFR and a timely delayed exogenous activation of EGFR. The latter is due to a c-Src- and metalloproteinase-mediated ectodomain shedding of cell surface-bound growth factors, which subsequently bind to the EGFR extracellular domain (ECD). Whereas 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and other dioxin-like compounds (DLCs) induce exactly the same signaling events, these chemicals somehow interfere with the growth factor-triggered EGFR activation. Further analyses revealed that DLCs directly bind to the ECD of EGFR, thereby displacing EGF and other polypeptide growth factors. Accordingly, treatment of EGFR-positive epithelial cells with (in terms of CYP1A1 induction) equipotent concentrations of PAHs and DLCs causes differences in signal transduction kinetics and ultimately gene expression patterns.

Here we expanded our study beyond the spectrum of environmental AHR ligands and investigated whether various other persistent organic pollutants of very high concern, including pesticides, flame retardants and UV filters, are capable of interacting with EGFR and altering downstream signal transduction and cellular functions. By conducting EGFR internalization assays, EGF-EGFR AlphaLISA assays, SDS-PAGE/Western blot analyses and BrdU incorporation assays, we identified several UV-stabilizing phenolic benzotriazoles and flame- retarding polybrominated diphenyl ethers to interfere with the binding of EGF to the EGFR ECD in a reasonable concentration range. The resulting inhibition of EGFR activity affected downstream MEK/ERK signal transduction and associated DNA synthesis. Data from *in silico* docking analyses and the subsequent mutation of the potentially involved amino acids strongly indicate that the EGFR serves as a receptor molecule for a broad variety of both 'AHR-active' as well as 'AHR-inactive' persistent organic pollutants.

In conclusion, our data identify the EGFR as a cell-surface receptor for environmental pollutants of very high concern and a critical determinant of the AHR response that may not only contribute to ligand- but also cell type- or tissue-specific differences in outcome.

[P72] Unique and common agonists activate the insect juvenile hormone receptor and the human AHR

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Transcription factors of the bHLH-PAS family play vital roles in animal development, physiology, and disease. Two members of the family require binding of low-molecular weight ligands for their activity: the vertebrate aryl hydrocarbon receptor (AHR) and the insect juvenile hormone receptor (JHR). In the fly *Drosophila melanogaster*, the paralogous proteins GCE and MET constitute the ligand-binding component of JHR complexes. GCE/MET represent the only known example of bHLH-PAS proteins acting as hormone receptors, and genes encoding them are absent from vertebrates (Tumova et al, 10.1016/j.jmb.2023.168332). Whilst GCE/MET and AHR are phylogenetically heterologous, their mode of action is similar. JHR is targeted by several synthetic agonists that serve as insecticides disrupting the insect endocrine system. AHR is an important regulator of human endocrine homeostasis and it responds to environmental pollutants and endocrine disruptors. Whether AHR signaling is affected by compounds that can activate JHR has not been reported. To address this question, we screened a chemical library of 50,000 compounds to identify 93 novel JHR agonists in a reporter system based on Drosophila cells. Of these compounds, 26% modulated AHR signaling in an analogous reporter assay in a human cell line, indicating a significant overlap in the agonist repertoires of the two receptors. To explore the structural features of agonist-dependent activation of JHR and AHR, we compared the ligand-binding cavities and their interactions with selective and common ligands of AHR and GCE. Molecular dynamics modeling revealed ligand-specific as well as conserved side chains within the respective cavities. Significance of predicted interactions was supported through site-directed mutagenesis. The results have indicated that synthetic insect juvenile hormone agonists might interfere with AHR signaling in human cells.

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TRAVEL GRANTS

EUROTOX travel grant

Gaëlle Deval, Université Paris Cité, Paris, France

Martina Karasová, Czech Academy of Sciences, Brno, Czech Republic

German Society for Environmental Mutation Research (GUM) travel grant

Darius Stukas, Lithuanian University of Health Sciences, Kaunas, Lithuania

Society for Free Radical Research Europe (SFRR-E) travel grant

António B. Pimpão, Universidade NOVA de Lisboa, Lisboa, Portugal

Travel fellowship provided by the organizers

Jeffry S. Tang, Malaghan Institute of Medical Research, Wellington, New Zealand

NOTES

This meeting is supported by the Deutsche Forschungsgemeinschaft (DFG).

Deutsche Forschungsgemeinschaft
German Research Foundation

In addition, we are grateful to the following companies and organizations for supporting the 6th International AHR Meeting in Düsseldorf!

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