

ISFMS
2025
Conference

The 5th International Symposium on Frontiers in Molecular Science

26–29 August 2025 | Kyoto, Japan



Notice

Delegates will receive a name-badge at the Information Desk upon registration. The badge must be worn prominently in order to gain access to the conference lecture halls and meals during the event. Admission will be refused to anyone not in possession of an appropriate badge.

Wi-Fi

Free Wi-Fi will be available at the conference venue.
The password will be provided on the day of the event.

Please note that connection quality within the university may vary.
If you plan to use mobile data, please ensure your device and data plan are prepared in advance.

Conference Photo

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The *International Journal of Molecular Sciences* (ISSN 1422-0067; CODEN: IJMCFK; ISSN 1661-6596 for printed edition) provides an advanced forum for molecular studies in biology and chemistry, with a strong emphasis on molecular biology and molecular medicine. Our aim is to provide rigorous peer review and enable rapid publication of cutting-edge research to educate and inspire the scientific community worldwide.

We encourage scientists to publish their experimental, theoretical, and computational results in as much detail as possible, in a regular section (<https://www.mdpi.com/journal/ijms/sections>) or in a Special Issue (https://www.mdpi.com/journal/ijms/special_issues). Therefore, there is no restriction on the maximum length of the papers or the number of electronic multimedia and supplementary files. For all articles, the full experimental details must be provided so that the results can be reproduced. Electronic files regarding the full details of the experimental procedure, if unable to be published in a normal way, can be deposited as supplementary material (including animated pictures, videos, interactive Excel sheets, software executables and others).

Manuscript categories: original scientific research articles, comprehensive reviews, communications, case reports, letters, commentaries, editorials, etc.

Impact Factor: 4.9 (2024); **5-Year Impact Factor:** 5.7 (2024)

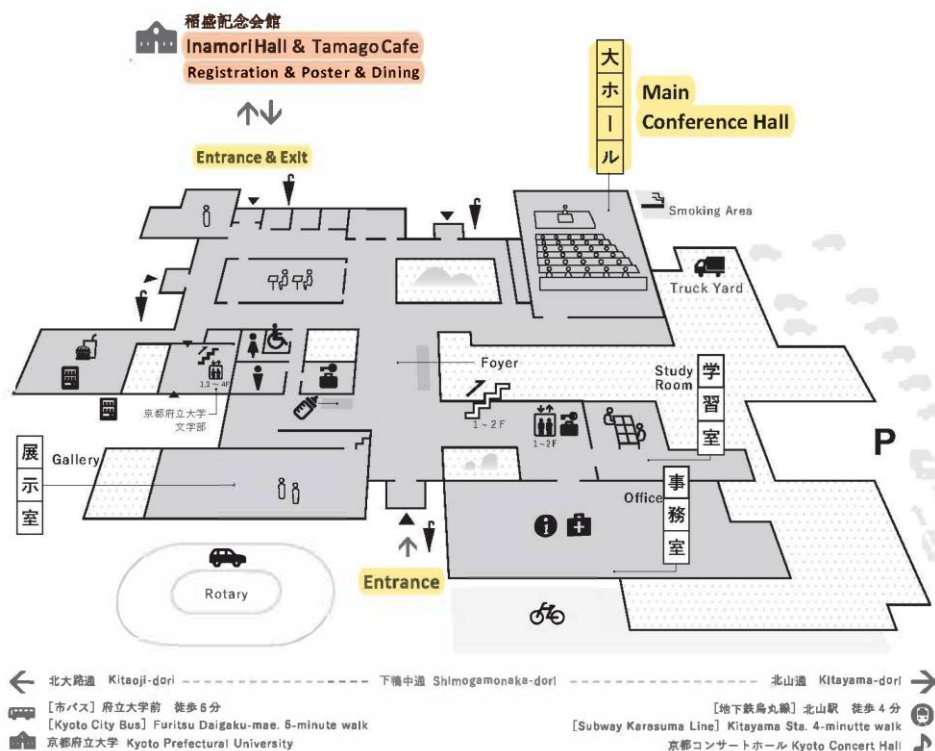
Venue

Address: Kyoto Institute, Library and Archives, Rekisaikan

Main Conference Hall
Rekisaikan



- Elevator
- Restroom
- Accessible Toilets
- Locker
- First Aid Room
- Nursing Room
- Parking (Paid)
- Bicycle Parking
- Umbrella Stand



Access and Surroundings

Rekisaikan and Inamori Hall are located in the northeastern part of Kyoto City, in Sakyo Ward, and are conveniently situated just a 6-minute walk from Exit 1 of Kitayama Station on the Kyoto City Subway Karasuma Line.

The area surrounding the venue is rich in academic and cultural resources, including Kyoto Prefectural University's Shimogamo Campus, Kyoto Prefectural Medical University, and the Kyoto Botanical Garden. Additionally, attendees will have the opportunity to explore the Shimogamo Shrine, a UNESCO World Heritage Site, and its surrounding Tadasu no Mori (Tadasu Forest). These attractions provide a refreshing natural and historical atmosphere, offering participants a chance to experience the cultural essence of Japan during their visit. This exceptional venue, deeply rooted in Japanese culture and history, will serve as the ideal setting for ISFMS2025.

Route to the Venue

After arriving at **Kansai International Airport (KIX)**, you can reach Kyoto and Inamori Hall either by train or by bus.

Recommended Option: By Train (Approx. 1 hour 50 minutes)

Step 1:

From Kansai Airport Station, take the JR HARUKA limited express train to Kyoto Station.

The Haruka runs approximately every 30 minutes (about two trains per hour), so you will not need to wait long for the next departure.

Step 2:

At Kyoto Station, transfer to the Subway Karasuma Line (bound for Kokusaikaikan). Get off at Kitayama Station and take Exit 1.

Follow the signs for "Subway Karasuma Line" after exiting the JR Central Gate; the route to the subway is clearly marked.

Step 3:

From Kitayama Station, it is about a 10-minute walk to Inamori Hall (Liberal Arts and Science Building).

By Bus (2 hours and 50 minutes)

Step 1:

From Terminal 1: Take Airport Shuttle Bus Number 8 to Kyoto Station (Hachijo-guchi).

From Terminal 2: Take Airport Shuttle Bus Number 2 to Kyoto Station (Hachijo-guchi).

Step 2:

Option A— Walk 10 minutes to Kyoto Station's City Bus Station (Bus Platform 2). Take City Bus No. 4 (bound for Nishigamo Shako, via Shijo Kawaramachi and Kamigamo Jinja Shrine) and alight at Kitanonochō. Finally, walk 7 minutes to Inamori Hall, Liberal Arts and Science Building.

Option B— From Kyoto Station (Hachijo-guchi), take a taxi to Inamori Hall. The ride takes about 22 minutes and costs approximately 1,800 JPY.

General Information

CONFERENCE VENUE

Session Venue:

Kyoto Institute, Library and Archives, Rekisaikan

Address: 1-29 Shimogamo Hangicho, Sakyo-ku, Kyoto City, Kyoto 606-0823



Source: <https://rekisaikan.jp/about/>, Kyoto Institute, Library and Archives, Rekisaikan (official website)

The venue for ISFMS2025 will be the Kyoto Institute, Library and Archives (Rekisaikan). This facility was established as an extension of the Kyoto Prefectural General Archives, which has been dedicated to the comprehensive collection, preservation, and public access of materials related to Kyoto for over 50 years. Rekisaikan was created to further enhance its role by incorporating research support, learning opportunities, and cultural exchange functions related to Kyoto's history and culture. Its modern yet serene design provides an ideal environment for research and discussion, ensuring a comfortable and productive experience for all participants of the conference.

Registration, Poster Session & Dining Venue:

Inamori Hall, Institute of Liberal Arts and Sciences

Kyoto Institute of Technology, Kyoto Prefectural University, Kyoto Prefectural University of Medicine

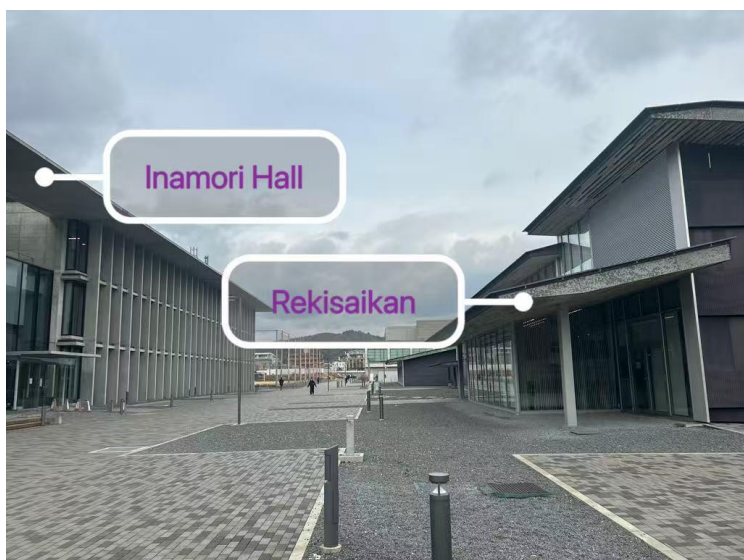


Source: <https://kyoto3univ.jp/>, Institute of Liberal Arts and Sciences (official website)

COLLABORATION And ACCESSIBILITY BETWEEN REKISAIKAN And INAMORI HALL

Rekisaikan is closely connected with Inamori Hall, located just across the road. The two facilities are within a very short walking distance of each other, ensuring optimal convenience for attendees. Inamori Hall was established by Kyoto Prefecture as a hub for interdisciplinary education, and it plays a vital role in fostering academic collaboration.

Notably, the Inamori Exhibition Room, located within Inamori Hall, highlights the philosophy and contributions of Kazuo Inamori. This exhibition provides a unique opportunity to reflect on the social and ethical significance of scientific research. The entire program of ISFMS2025 will be hosted across both Rekisaikan and Inamori Hall. Their proximity greatly reduces transit time between sessions, enabling a seamless and efficient conference experience.



CERTIFICATE of ATTENDANCE

Event participants will be able to download an electronic Certificate of Attendance by accessing their dashboard on Sciforum.net once the event is concluded. The certificate will be found under the “My certificates” category.

INSURANCE

Organizers are not liable for personal accidents or property loss.

The 5th International Symposium on Frontiers in Molecular Science

26–29 August 2025 | Kyoto, Japan



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Welcome from the Chairs

Dear Colleagues,

We cordially invite you to participate in the **5th International Symposium on Frontiers in Molecular Sciences (ISFMS2025)—Molecular Regulatory Mechanisms of Biological Function and Drug Discovery based on Protein Structure/Function Analysis**, to be held in Kyoto, Japan, from August 26 to 29, 2025. The symposium will present outstanding research results that elucidate the molecular mechanisms of biological function and regulation, and it will also facilitate drug design based on molecular biology, biophysical characterization, in vivo environmental homeostasis, organ interactions based on sensory systems, physiome, and AI-assisted analysis of protein structures and functions.

The venue will be the Inamori Hall, Liberal Arts and Science Building and the Kyoto Institute, Library and Archives (Rekisaikan), both affiliated with Kyoto Prefectural University, Kyoto Prefectural University of Medicine and the Kyoto Prefectural Government, located in Kyoto City. The venue is conveniently located approximately 20 minutes by subway or car from Japan Railway (JR) Kyoto Station, which is also easily accessible from Kansai International Airport (KIX) by JR (approximately 1 hour 20 minutes) or limousine bus (approximately 1 hour 30 minutes).

Kyoto, the ancient capital of the millennium, has a fascinating historical heritage and features numerous World Cultural Heritage sites, including Kiyomizu-dera Temple, Nijo-jo Castle, Shimogamo-Jinja Shrine, Kinkaku-ji Temple, Ginkaku-ji Temple, Saiho-ji (Koke-dera) Temple and the Kozan-ji Temple, among others. These sites will be of great interest to visitors. Gion is another renowned location in Kyoto, showcasing the city's rich heritage and traditional Japanese culture. You can immerse yourselves in the authentic Japanese dances and music performed by Geiko and Maiko artists.

We look forward to welcoming you to Kyoto Japan.



Dr. Masayuki Takahashi
Conference Chair

1. Kyoto Prefectural University, Japan;
2. Institute of Science Tokyo, Japan



Prof. Dr. Akiyuki Taruno
Conference Chair
Kyoto Prefectural University
of Medicine, Japan



Prof. Dr. Atsushi Shiozaki
Conference Chair
Kyoto Prefectural University
of Medicine,
Japan



Prof. Dr. Yoshinori Marunaka
Conference Chair
1. Kyoto Industrial Health Association,
Japan; 2. Kyoto Prefectural University of
Medicine, Japan; 3. Ritsumeikan Uni-
versity, Japan



Prof. Dr. Yusaku Iwasaki
Conference Chair
Kyoto Prefectural University,
Japan

Local Organizing Committee

Local Organizing Committee Chair



Prof. Dr. Takashi Nakahari
Ritsumeikan University, Japan

Local Organizing Committee Member



Prof. Dr. Kikuko Amagase
Ritsumeikan University, Japan



Prof. Dr. Motoyuki Iemitsu
Ritsumeikan University, Japan



Prof. Dr. Naomi Niisato
Kyoto University of Advanced
Science, Japan



Prof. Dr. Takeshi Hashimoto
Ritsumeikan University, Japan



**Prof. Dr. Tomoe Y.
Nakamura-Nishitani**
Wakayama Medical University,
Japan



Prof. Dr. Tsukasa Okiyonedo
Kwansei Gakuin University,
Japan

Scientific Committee



Dr. Alexandre G. De Brevern
Necker-Enfants Malades Hos-
pital, France



Dr. Andrea Ferrigno
University of Pavia, Italy



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The University of Texas Rio
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University of Genoa, Italy



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Kyoto Prefectural University of
Medicine, Japan



Prof. Dr. Antonello Merlino
University of Naples Federico II, Italy



Prof. Dr. Assaf Friedler
The Hebrew University of Jeru-
salem, Israel



Prof. Dr. Atsushi Shiozaki
Kyoto Prefectural University of
Medicine, Japan



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University of Electronic Science and Technology of China, China



Prof. Dr. Jesus Vicente De Julian-Ortiz
University of Valencia, Spain



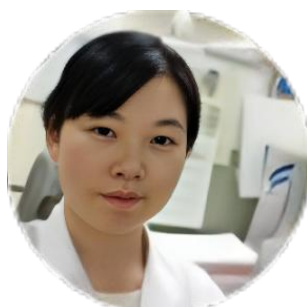
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Dalian University of Technology, China



Prof. Dr. Josef Jampilek
Comenius University, USA



Prof. Dr. Katsunori Nonogaki
Tohoku University, Japan



Prof. Dr. Keiko Hosohata
Osaka University of Pharmaceutical Sciences, Japan



Prof. Dr. Luca Roncati
Link Campus University, Italy



Prof. Dr. Masayuki Takahashi
1. Kyoto Prefectural University,
Japan; 2. Institute of Science To-
kyo, Japan



Prof. Dr. Rony Seger
Weizmann Institute of Science,
Israel



Prof. Dr. Victor Muñoz
University of California
Merced, USA



Prof. Dr. Yoshihiro Kubo
National Institute for Physiologi-
cal Sciences, Japan



Prof. Dr. Yoshinori Marunaka
1. Kyoto Industrial Health Associa-
tion, Japan; 2. Kyoto Prefectural
University of Medicine, Japan; 3.
Ritsumeikan University, Japan



Prof. Dr. Yury O. Chernoff
Georgia Institute of Technol-
ogy, USA



Prof. Dr. Yusaku Iwasaki
Kyoto Prefectural University,
Japan

Session Chairs



Dr. Masayuki Takahashi
1. Kyoto Prefectural University,
Japan; 2. Institute of Science To-
kyo, Japan



Prof. Dr. Antonello Merlino
University of Naples Federico II,
Italy



Prof. Dr. Atsushi Shiozaki
Kyoto Prefectural University of
Medicine, Japan



Prof. Dr. Billy K.C. Chow
The University of Hong Kong,
China



Prof. Dr. Maria Hrmova
University of Adelaide,
Australia



Prof. Dr. Sharon Y. C. Ruan
The Hong Kong Polytechnic Uni-
versity, China



Prof. Dr. Yoshinori Marunaka
1. Kyoto Industrial Health Associ-
ation, Japan; 2. Kyoto Prefectural
University of Medicine, Japan;
3. Ritsumeikan University, Japan



Prof. Dr. Yusaku Iwasaki
Kyoto Prefectural University,
Japan

Plenary Speakers



Prof. Dr. Harry Brumer

University of British Columbia, Canada

Prof. Dr. Brumer works in the area of fundamental and applied carbohydrate enzymology and biochemistry. His research interests include the discovery, functional and structural characterization, and biotechnological applications of carbohydrate-active enzymes and carbohydrate-binding proteins. A key focus of the Brumer Group is to illuminate the molecular mechanisms of complex carbohydrate (“dietary fibre”) utilization in the human gut microbiota, and to harness this emergent knowledge to improve health.



Prof. Dr. Hideki Sakai

University of Toyama, Japan

Professor Hideki Sakai is a distinguished researcher and educator at the University of Toyama, specializing in Pharmaceutical Physiology. With a Ph.D. in Pharmaceutical Sciences from Toyama Medical and Pharmaceutical University (1992), he has made significant contributions to the field of cell physiology, focusing on ion-transporting proteins in epithelial cells. His research employs physiological, biochemical, and pharmacological techniques. Prof. Sakai has an impressive academic record with 2501 citations and an h-index of 25. He has been involved in multiple research projects and has published extensively, with 91 articles and 8 review papers. His expertise spans cell physiology, electrophysiology, and biophysical chemistry.



Prof. Dr. Akiyuki Taruno

Kyoto Prefectural University of Medicine, Japan

His lab investigates the neural basis of epithelial chemosensations in the peripheral nervous systems and their processing in the central nervous system by employing an array of techniques, including optical imaging, electrophysiological recording, optogenetic manipulation, single-cell transcriptomics, and behavioral assays. He started his research in gustation as a post-doc at the University of Pennsylvania in the Department of Physiology in 2010 before moving to the Kyoto Prefectural University of Medicine in the Department of Molecular Cell Physiology as an assistant professor in 2013 (Professor 2018–present). His previous studies have helped identify key components of peripheral taste transduction. They include the molecular mechanisms of how taste cells lacking synaptic vesicles release neurotransmitters and how sodium taste is processed within taste cells. These findings have helped establish the existence of a novel chemical synapse (dubbed the channel synapse), which provides a new paradigm for understanding the mechanisms of epithelium–neuron chemical communication.

Keynote Speakers



Dr. Alessio Nocentini
University of Florence,
Italy



Dr. (Chris) Lok-To Sham
National University of
Singapore, Singapore



Dr. Luigi Vitagliano
National Research Council,
Italy



Dr. Masayuki Takahashi
1. Kyoto Prefectural University, Ja-
pan; 2. Institute of Science Tokyo,
Japan



Prof. Dr. Antonello Merlino
University of Naples Federico II,
Italy



Prof. Dr. Atsushi Shiozaki
Kyoto Prefectural University
of Medicine, Japan



Prof. Dr. Leila Lo Leggio
University of Copenhagen,
Denmark



Prof. Dr. Maria Hrmova
University of Adelaide,
Australia



Prof. Dr. Masahiro Ohsawa
Teikyo University, Japan



Prof. Dr. Sharon Y. C. Ruan
The Hong Kong Polytechnic
University, China



Prof. Dr. Tsukasa Okiyonedo
Kwansei Gakuin University,
Japan



Prof. Dr. Victor Muñoz
University of California
Merced, USA



Prof. Dr. Yoichiro Isohama
Tokyo University of Science, Japan



Prof. Dr. Yusaku Iwasaki
Kyoto Prefectural University, Japan

Invited Speakers



Dr. Hiroki Shimizu
Kyoto Prefectural University of
Medicine, Japan



Dr. Kazunari Miyamichi
RIKEN Center for Biosystems Dy-
namics Research, Japan



Dr. Yuta Masuda
Kyoto Prefectural University,
Japan



Assoc. Prof. Masahiro Nakajima
Tokyo University of Science,
Japan



Assoc. Prof. Dr. Laura Masgrau
Autonomous University of Barce-
lona, Spain



Prof. Dr. Antoni Planas
University Ramon Llull,
Spain



Prof. Dr. Assaf Friedler
The Hebrew University of Jerusalem,
Israel



Prof. Dr. Edward L. D'Antonio
University of South
Carolina Beaufort, USA



Prof. Dr. Jean-Didier Maréchal
Autonomous University of Barce-
lona, Spain



Prof. Dr. Kento Kurashima
Division of Digestive Surgery, Kyoto
Prefectural University of Medicine,
Japan

Program Overview

	26 August 2025 Tuesday	27 August 2025 Wednesday	28 August 2025 Thursday	29 August 2025 Friday
Morning	The venue opens at 9:00 every day.	Registration & Welcome Coffee (8:30–9:30)	Registration (8:30–9:15)	Registration (8:30–9:15)
		Opening Cere- mony	Session 1	Session 4
		Session 2	Coffee Break & Poster Session	Coffee Break
		Lunch Break	Session 1	Session 4
			Lunch Break	Lunch Break
Afternoon	Registration (15:00–18:00)		Session 2	Session 1
		Coffee Break & Poster Session	Coffee Break & Poster Session	Coffee Break
		Session 5	Session 3	Session 6
				Award Ceremony & Closing Re- marks
	/	Finger Food Party	/	/

ISFMS 2025 Program

27 August 2025 (Wednesday)
Session 2. Chaired by Prof. Dr. Maria Hrmova
Time: 8:30 (JST, Tokyo) | Main Venue (Rekisaikan)

JST Time	Speaker	Title
8:30–9:30		Registration & Welcome Coffee
9:30–9:40		Opening Ceremony and Welcome Speech from the Event Chair: Prof. Dr. Yoshinori Marunaka
		Session 2. Chaired by Prof. Dr. Maria Hrmova
9:40–10:10	Maria Hrmova Keynote Speaker	X-ray crystallography united with computational modelling reveal the catalytic mechanism in a hydrolytic enzyme
10:10–10:40	Leila Lo Leggio Keynote Speaker	LsAA9A Lytic Polysaccharide Monooxygenase: from initial structures to advanced crystallography of a model copper enzyme
10:40–11:00	Masahiro Nakajima Invited Speaker	Finding of phylogenetically, functionally, structurally, and mechanistically new glycoside hydrolase family enzymes acting on β -1,2-glucans
11:00–11:20	Laura Masgrau Invited Speaker	Computational studies on carbohydrate-active enzymes
11:20–11:35	Zhiqi Cong Oral Presenter	Engineering hydrogen peroxide tunnel for generating non-natural P450 peroxygenases
11:35–11:50	Piyanut Pinyou Oral Presenter	Bi-Enzymatic Biocatalytic System of β -Glucosidase (TxGH116)/Glucose Oxidase for a Cellobiose/H ₂ O ₂ Biofuel Cell
11:50–12:05	Anastasia A. Pometun Oral Presenter	Structure-functional main features of the enzymes, expressed by lactobacilli in response to Klebsiella
12:05–13:30		Lunch Break

Session 2. Chaired by Prof. Dr. Maria Hrmova
Session 5. Chaired by Dr. Masayuki Takahashi
Time: 13:00 (JST, Tokyo) | Main Venue (Rekisaikan)

JST Time	Speaker	Title
13:30–14:15	Harry Brumer Plenary Speaker	The human gut microbiota-plant cell wall nexus: Enzyme discovery and applications
		Session 2. Chaired by Prof. Dr. Maria Hrmova
14:15–14:35	Antoni Planas Invited Speaker	Chitin deacetylases for the production of sequence-defined chitosan oligosaccharides. Understanding and Engineering specificity
		Session 5. Chaired by Dr. Masayuki Takahashi
14:35–15:05	Masayuki Takahashi Keynote Speaker	Drug design for the solution to drug resistance
15:05–16:00		Coffee Break & Poster Session
		Session 5. Chaired by Dr. Masayuki Takahashi
16:00–16:30	Alessio Nocentini Keynote Speaker	Multitarget Drug Design for Complex Diseases: Focus on Carbonic Anhydrase Modulators
16:30–16:50	Assaf Friedler Invited Speaker	Intrinsically disordered proteins as drug targets
16:50–17:05	Daniil R. Bazanov Oral Presenter	Targeting p53-MDM2/MDMX Interaction: Advances in Nutlin Development and Therapeutic Applications
17:05–17:20	Alisa Sokolovskaya Oral Presenter	Simulated microgravity induced changes to drug response, increasing heat shock proteins expression but not apoptosis markers in megakaryoblastic cell line
17:20–17:35	Mingxin Su Oral Presenter	IL-15 Blocks Autophagic Flux in Pancreatic Stellate Cells to Enhance Alanine Secretion and Sustain Pancreatic Cancer Gemcitabine Resistance
17:35–17:50	Qi Tan Oral Presenter	Discovery Of Moracin D As A Novel Interleukin-1 β Inhibitor To Alleviate Acute Lung Injury
17:50–18:05	Wally Ibatuan Tapas Oral Presenter	In Silico Analysis of Antibacterial Phenylphenalonones from Musa textilis (Abaca) Pseudostem: A Molecular Docking Approach
18:30–20:00		Finger Food Party

28 August 2025 (Thursday)**Session 1. Chaired by Prof. Dr. Antonello Merlino****Time: 8:30 (JST, Tokyo) | Main Venue (Rekisaikan)**

JST Time	Speaker	Title
8:30–9:15		Registration

Session 1. Chaired by Prof. Dr. Antonello Merlino

9:15–9:45	Antonello Merlino Keynote Speaker	Protein metalation by vanadium-based drugs
9:45–10:15	Luigi Vitagliano Keynote Speaker	Unravelling structure-function relationships in KCTD proteins
10:15–10:30	Faisal Koua Oral Presenter	Sub-ångström resolution structure of an iron-sulfur protein resolved using serial femtosecond crystallography
10:30–10:45	Ayub Haree Oral Presenter	Generalised Protein Backmapping with Deep Learning (CNNs): From Coarse-Grained to Atomistic
10:45–11:00	Xiakun Chu Oral Presenter	Transcription factor clustering shapes chromatin dynamics to regulate enhancer-promoter interactions
11:00–11:30		Coffee Break & Poster Session
11:30–12:00	Victor Muñoz Keynote Speaker	Lessons about Protein Folding, Binding, and Intrinsic Disorder from the Study of Archetypal Protein Folds
12:00–12:15	Giarita Ferraro Oral Presenter	Metallodrugs Encapsulation Within Ferritin Nanocages
12:15–12:30	Julien Hugo Mignon Oral Presenter	Influence of environmental changes on the conformational and amyloidogenic landscapes of an IDP: a combined biophysical and molecular dynamics investigation
12:30–13:30		Lunch Break

Session 1. Chaired by Prof. Dr. Antonello Merlino**Session 3. Chaired by Prof. Dr. Sharon Y. C. Ruan****Time: 13:30 (JST, Tokyo) | Main Venue (Rekisaikan)**

JST Time	Speaker	Title
13:30–14:15	Hideki Sakai Plenary Speaker	Novel Insights into Intracellular P-Type ATPase Functions in Disease

Session 1. Chaired by Prof. Dr. Antonello Merlino

14:15–14:35	Edward L. D'Antonio Invited Speaker	Discovery of Antitrypanosomal Gossypol Derivatives Targeting the Glucose Kinases: Insights from Biochemical, Molecular Docking, and In Vitro Biological Methods
14:35–14:55	Jean-Didier Maréchal Invited Speaker	Multiscale molecular modeling of metalation processes in proteins: from recognition to catalysis
14:55–15:10	Florian Kongoli Oral Presenter	FLOGEN NEW SUSTAINABILITY FRAMEWORK AND THE ROLE OF MOLECULAR SCIENCES AND DRUG DEVELOPMENT
15:10–15:25	Davis Joseph Oral Presenter	The Unified Theory of Neurodegeneration Pathogenesis Based on Axon Deamidation
15:25–16:00		Coffee Break & Poster Session

Session 3. Chaired by Prof. Dr. Sharon Y. C. Ruan

16:00–16:30	Tsukasa Okiyoneda Keynote Speaker	Protein quality control mechanisms of CFTR as therapeutic targets for cystic fibrosis.
16:30–17:00	Sharon Y. C. Ruan Keynote Speaker	Novel roles of epithelial ion channels in endocrine functions
17:00–17:15	Yair Ben-Chaim Oral Presenter	Voltage dependence of G protein coupled receptors
17:15–17:30	Yuka Kamada Oral Presenter	Functional analysis of HECT-type E3 ligase HERC3 in ER-associated degradation of CFTR mutants
17:30–17:45	Hoi Leong Xavier Wong Oral Presenter	MT1-MMP: new therapeutic target of metabolic disorder

29 August 2025 (Friday)**Session 4. Chaired by Prof. Dr. Atsushi Shiozaki and Prof. Dr. Yoshinori Marunaka****Time: 8:30 (JST, Tokyo) | Main Venue (Rekisaikan)**

JST Time	Speaker	Title
8:30–9:15	Registration	
Session 4. Chaired by Prof. Dr. Atsushi Shiozaki and Prof. Dr. Yoshinori Marunaka		
9:15–9:45	Atsushi Shiozaki Keynote Speaker	Ion Channel Profiling and Therapeutic Targeting in Cancer Stem Cells of Digestive Malignancies
9:45–10:15	Masahiro Ohsawa Keynote Speaker	Application of Kampo medicines for the palliation of cancer cachexia.
10:15–10:35	Hiroki Shimizu Invited Speaker	The potential of ion channels as therapeutic targets in gastrointestinal cancers
10:35–10:50	Sitthinon Siripanthong Oral Presenter	Using Adhiron technology to develop PAK1-selective inhibitors for targeted cancer treatment
10:50–11:05	Rafaële Calvo Esposito Oral Presenter	New insights in the proteolytic contribution of Ananas comosus extract to its in vitro anti-cancer effects against colon cancers
11:05–11:20	Coffee break	
11:20–11:50	Yoichiro Isohama Keynote Speaker	Novel functions of aquaporins in cancer and their potential as therapeutic targets
11:50–12:05	Akira Ikari Oral Presenter	Inhibition of proliferation of colorectal cancer cells by suppression of claudin-14 expression
12:05–12:20	Oskar Kruc Oral Presenter	Design of potent elongated m-Terphenylpicolinamide derivatives and their interactions with the PD-L1 protein
12:20–12:35	Motoki Watanabe Oral Presenter	Identification of ANT2 as a Druggable Target for Endocrine-Resistant ERα-Positive Breast Cancer
12:35–13:30	Lunch Break	
Session 4. Chaired by Prof. Dr. Atsushi Shiozaki and Prof. Dr. Yoshinori Marunaka		
Session 6. Chaired by Prof. Dr. Yusaku Iwasaki and Prof. Dr. Yoshinori Marunaka		
Time: 13:30 (JST, Tokyo) Main Venue (Rekisaikan)		
JST Time	Speaker	Title
13:30–14:15	Akiyuki Taruno Plenary Speaker	Discovery of the channel synapse and its biological functions
Session 4. Chaired by Prof. Dr. Atsushi Shiozaki and Prof. Dr. Yoshinori Marunaka		
14:15–14:35	Kento Kurashima Invited Speaker	The role of ferroptosis in gastrointestinal cancers and development of novel therapeutics targeting ferroptosis
14:35–15:05	Coffee break	
Session 6. Chaired by Prof. Dr. Yusaku Iwasaki and Prof. Dr. Yoshinori Marunaka		
15:05–15:35	Yusaku Iwasaki Keynote Speaker	Gut-derived glucagon-like peptide-1 induces satiation beneficial for obesity prevention
15:35–16:05	Lok-To Sham Keynote Speaker	Profiling the Genetic Interaction Landscape in Bacteria
16:05–16:25	Kazunari Miyamichi Invited Speaker	Labeled-Line Circuits of Molecularly-Delineated Sympathetic Neurons for Selective Organ Regulation
16:25–16:45	Yuta Masuda Invited Speaker	Gut hormone cholecystokinin drives oxytocin-dependent thermogenesis in brown adipose tissue via vagal sensory nerve–brain axis.
16:45–17:00	Ryusuke Yoshida Oral Presenter	The roles of chemical synapses in mouse taste bud cells
17:00–17:15	Takashi Nakahari Oral Presenter	Ryu-Kaku-San, a herbal medicine, enhances ciliary microbeads transport on ciliated human bronchial epithelia via TRPV4 activation: propagation of metachronal wave
17:15–17:30	Sharifah Zamiah Syed Abdul Kadir Oral Presenter	Des-aspartate Angiotensin 1 (DAA-1) as a Novel AT1R Modulator: In-Silico and Vascular Functional Insights
17:30–17:45	Yu-Kuo Chen Oral Presenter	Acetylated derivative of nobletin may alleviate Alzheimer-like cognitive impairment through PI3K/AKT signaling pathway and gut microbiota in mice
17:45–18:00	Award Ceremony & Chair Closing Remarks	

Poster List

Day 2–27 August 2025 (Wednesday) | 15:05–16:00

Posters should be set up during lunch break and before 14:00 and must be removed after finger food party on the same day.

Day 3–28 August 2025 (Thursday) | 11:00–11:30 & 15:25–16:00

Posters should be set up from 8:30 to 9:15 and must be removed by 15:50 on the same day

27 August 2025		
1	Toshio Hattori	Decomposed matricellular proteins reflect the immune-protease activities of COVID-19
2	Wanda Destiarani	Computational Insights into the pH-Dependent Behavior of Ipilimumab-CTLA-4: A Switchable Antibody
3	Harshika Mahaveerchand	Structure-Guided Analysis and Functional Characterization of a Plant-Derived Coagulant Protein for Synthetic Dye Bioremediation
4	Joshua Lam	Exploring ATP Non-Specific Behaviour: How Forcefield Choice Shapes Molecular Dynamics Outcomes
5	Nelli Muradyan	Combining AlphaFold2 and MD Simulations to Elucidate 14-3-3-Mediated Assembly of IDR-Containing Proteins
6	Gabriella Tito	Interaction of new mixed-valence Polyoxovanadate clusters with proteins
7	Noah James Graves	GFP-Paint: Using Binding Affinity of GFP-Tagged HSPs to Strainotype alpha-Synuclein Polymorphs
8	Cong Liu	Development of an α -synuclein PET tracer for clinical diagnosis of Parkinson's diseases
9	Dan Li	Structural polymorphism of α -synuclein amyloid fibrils in Parkinson's disease
10	Shengnan Zhang	Design of Ig-like binders targeting α -synuclein fibril for mitigating its pathology
11	Vangelis Daskalakis	Molecular Dynamics of RNA-Guided Endonucleases: Comparative Insights into Cas12 and Eukaryotic Fanzor Systems
12	Akifumi Oda	Computational studies for structural features of tetrameric amyloid β 1-42 peptides with D-Asp1, 7, and 23 residues
13	Khushboo Bhagat	Multiscale Simulations and Profiling of Human Thymidine Phosphorylase Mutations: Insights into Structural, Dynamics, and Functional Impacts in Mitochondrial Neurogastrointestinal Encephalopathy
14	Rosanna Lucignano	New nanomaterials based on bioactive peptides: structural studies and molecular dynamics
15	Priya Dey	Caffeine as an Inhibitor of Amyloid- β Dimerization: A 3D-RISM and Molecular Dynamics Approach
16	Janusz Blasiak	Histone deacetylases 1 and 6 in migraine: potential therapeutic targets
17	Felipe Avila	Thermal treatment of Ribes magellanicum reduces the polyphenol-mediated protection of the proteasome and increases the accumulation of glycated proteins in AGS cells exposed to methylglyoxal
18	Wei yi	Construction of Rice Bran-Derived Glucosylceramide Liposomes and Their Application in Subcellular Enzyme Activity Analysis
19	Yuji Kanazawa	Effect of Skeletal Muscle Stretching on Factors Affecting Collagen Cross-linking
20	Safaa Omar Elazab Abdelhalim	Breaking down of plastic waste via insect enzymes

21	Vasil Pirgozliev	Gene expression of jejunal tight junction proteins in chickens fed supplementary myo-inositol
22	Vasil Pirgozliev	Effect of dietary phytase on gene expression of myo-inositol transporters in chicks jejunum
23	Luciranon Sribrama	Engineered-Enzyme Biomass Fuel Cells: Enhancing Bio-electrochemical Efficiency for Sustainable Energy
24	Hwa-Yeong Kim	Deubiquitinating enzymes associated with paclitaxel response in lung cancer cells
25	Sun-Kyu Jin	Differential expression of deubiquitinating enzymes in cisplatin-treated lung cancer cells
26	Supattra Poeaim	Natural Product-Based Discovery of Antibacterial Agents from <i>Sophora tomentosa</i> L. to Tackle Drug Resistance
27	Lexie Xiaoming CUI	Attenuation of Alzheimer's Disease-Associated Neuroinflammation Using Microglia-targeting Luteolin Nanoliposomes: A Proof-of-Concept Study
28	King-Ho CHEUNG	Luteolin Mitigates Neuroinflammation of Alzheimer's Disease Through the Activation of Mitophagy
29	Jane Liu	Design and Development Strategy for Target Proteins used in Antibody Drug Discovery
30	Ting Pan	The crosstalk between Ubiquitination and GlcNAcylation of CHAF1A regulates the HIV-1 latency and reactivation
31	Karakoz Zhailovna Badekova	Formulation of <i>Salvia dumetorum</i> Extract-Based Tablets for the Treatment of <i>Streptococcus mutans</i> Infections: Antimicrobial and Antibiofilm Properties
32	Nina Tsao	The AR-12 derivative P12-8 enhances autophagy in human microvascular endothelial cells against group A <i>Streptococcus</i> infection
33	Hung Huey-Shan	Characterization and Therapeutic Potential of Au-Col-Allicin Nanocomposite in DBTRG Glioblastoma Cells
34	Kritsada Pruksaphon	Integrated Computational Approaches Identified Gallic Acid-Flavonoid Conjugates as Effective Inhibitors against SARS-CoV-2 Main Protease (Mpro)
35	Kushal Bora	Development and evaluation of novel dual-target drugs against visceral leishmaniasis
36	Piotr Hanczyc	Lasing effect in studies on amyloid oligomers linked with neurodegenerative diseases
37	Krishna Yallappa Kolachi	Machine Learning Driven Techniques For The Discovery Of Nrf2/ARE Pathway Modulators For Combating Parkinson's Disease
38	Sana Inoue	The Role of Leukotriene B4 Type 2 Receptors in the Healing Process of Anticancer Agent-Induced Enteritis
39	YUN-RU WU	Probiotic <i>Lactobacillus rhamnosus</i> GG Attenuates PM2.5-induced Respiratory Inflammation and Modulates Cytokine Responses
40	Yuuki Otsuka	Dynamic Regulation of Zinc Transporters During Experimental Ileitis in Mice
41	Vladimir I. Tishkov	Formate dehydrogenase for detection of NAD ⁺ in biological samples
42	Swastika Banerjee	Comparative Docking and Systems-Level Terpenoid Integration Show Celastrol's Multi-Target Modulator in Parkinson's Pathophysiology

28 August 2025		
1	Shaocong Ou	Effects of Voltage Dependence and Rate Constants on Transient Currents in a Thermodynamically Constrained Mathematical Model of the Na/Ca Exchanger
2	Shani Stern	Dysregulation of Multiple Solute Carrier genes and Metabolic Deficits in SLC1A4-Mutant Human iPSC-Derived Hippocampal Neurons
3	Maggie Ming-Ki LEUNG	Targeting TRPM7 with Carvacrol to Alleviate Neuroinflammation in Alzheimer's Disease: Mechanistic and Therapeutic Insights
4	Kuntong Jia	Tetraspanin CD9 serves as a cellular receptor for fish Rhabdovirus eliciting clathrin- and caveolae-mediated endocytosis
5	Naoto Oikawa	Effect of γ -secretase dysfunction on neuronal cholesterol metabolism
6	Maika Miwa	Sirtuin-2 plays a key role in improving age-related decline in skin barrier function
7	Shing-Hwa Liu	Indoxyl sulfate, a uremic toxin, inhibits myoblast differentiation by the imbalance of cellular iron metabolism and reactive oxygen species
8	Yugo Nakazato	Integrins may regulate epithelial cell adhesion to cuticle for color pattern development in butterflies
9	Momo Ozaki	Involvement of TRP channels in the butterfly wing eyespot determination
10	David Y.B. Deng	Oxymatrine alleviates severe acute pancreatitis by inhibiting the excessive formation of NETs via the LFA-1/PKC/ROS pathway in neutrophils
11	Hae-Seul Choi	Deubiquitinating enzyme-mediated regulation of the pro-apoptotic protein Bax
12	Peng Zhu	Expression of epithelial-mesenchymal transition markers in circulating tumor cells from hepatocellular carcinoma patients after radical resection
13	Kuo-Lun Kuei	Synergistic Antitumor Effects and Underlying Mechanisms of Sodium Butyrate and Dovitinib Co-treatment in A549 Non-Small Cell Lung Cancer Cells
14	Kuo-Sheng Cheng	Antitumor effect and mechanism of 3-Methylindole and its derivative epoxydibenzonaphthyridine (O-DBNT) in MDA-MB-231 breast cancer cell line
15	Ning Wang	Infiltration of immunosuppressive myeloid population in hypoxic area of hepatocellular carcinoma determines its response to anti-PD1 therapy
16	Sooyeon Park	Panobinostat enhances 5-FU efficacy in gastric cancer cells through thymidylate synthase regulation
17	Nayeon Kim	Sodium butyrate improves 5-fluorouracil sensitivity in gastric cancer via thymidylate synthase and cell cycle regulation
18	Weiyi Tang	Major Advances and Limitations in CAR T cell Therapy
19	Pipin Agnesia	EPA and DHA from <i>Decapтерus macarellus</i> Suppresses Colorectal Cancer Cell Proliferation and Apoptosis via TP53 and NF- κ B Pathway Modulation
20	Zhu Zhang	20(S)-protopanaxadiol inhibits pancreatic cancer progression through targeting the C-X-C chemokine receptor type 4
21	Miao Yu	The Dynamic Feedback Between IL-6 and TWIST1 Drives Pancreatic Cancer Progression
22	Chih-Peng Chang	Sorafenib and regorafenib trigger IL-33-mediated immunosuppression to compromise the treatment efficacy in hepatocellular carcinoma
23	Jiann-Jyh Huang	Highly Expressed in Cancer 1 (Hec1): A Novel Therapeutic Target in Human Cancers
24	Po-Chang Shih	Structure-based drug design for targeting hard-to-target transcription factor STAT3 by focusing on a novel ligand binding site

25	Deisy Yurley Rodriguez-Sarmiento	Exploring the Signaling Mechanisms of Kisspeptin Receptor: A Structure-Activity Approach for Designing Safer Therapeutics
26	Jiayi Jin	Monitoring Heatstroke: A Proteomics-Based Multibiomarker Approach to Assess Potential Post-Heatstroke Complications
27	Kengo Iba	Vagal sensory neurons expressing oxytocin receptor regulate mental function via hypothalamic oxytocin neurons
28	Ken-ichi Aihara	Pemafibrate, a selective peroxisome proliferator-activated receptor α modulator, exerts eNOS-independent protective action against the development of steatotic liver disease in mice
29	Muyan Chu	Enhancing CFTR facilitates osteoporotic bone fracture healing in mice
30	Xiaojun Cai	Ovarian granulosa-specific knockout of multidrug resistance protein 4 (MRP4) induces female subfertility by disturbing late folliculogenesis and estrus cycle in mice
31	Sin-Jin Li	Targeting mitochondrial fission and extracellular mitochondria to ameliorate hepatic injury in metabolic dysfunction-associated fatty liver disease
32	Xin He	Histone chaperone CHAF1B facilitates germinal center B cell differentiation via cooperation with BCL6 and TBL1XR1
33	Shu-Wen Wan	Rab27-dependent release of extracellular vesicles facilitates the secretion of the dengue virus non-structural protein 1
34	Chih-Feng Kuo	Characterization of the content of endothelial extracellular vesicles derived from group A Streptococcus-infected human dermal microvascular endothelial cells
35	Yuki Shinohara	Regulation of angiotensinogen secretion by reactive oxygen species in mesangial cells
36	Masumi Kamiyama	Isoflavones regulate reactive oxygen species-induced angiotensinogen release in proximal tubule cells
37	Hazel Alexis Liang	Development of a Centella Asiatica - Based Antibacterial Spray to Inhibit the Growth of Listeria Monocytogenes on Food
38	TOMOHIRO SUGIYAMA	Prediction of Ischemic Stroke Using Serum Antibody Markers with a Machine Learning Model
39	Yu-Chun Wang	Illuminating Vitamin D's Regulatory Influence on Chloride Ion Dynamics in Zebrafish
40	Jumpei Yasuda	FUT8, a glycosyltransferase, mediates cardiac dysfunction during isoproterenol-induced heart failure mouse models
41	Sinisa Dovat	Casein Kinase II (CK2) regulates cell cycle progression and chromatin remodeling in hematological malignancies
42	Ting-Kuang Yeh	Explore the function of microRNAs in regulating the CaMKII α /SIRT1 signaling pathway and their implications for cognitive performance in adolescents
43	Jiping Zeng	The circ_0071616-miR-140-3p-USP34 axis mediates FoxM1 deubiquitination in Helicobacter pylori-induced gastric malignant transformation

Session 1. Protein Structure and Molecular Dynamics

sciforum-123091: Combining AlphaFold2 and MD Simulations to Elucidate 14-3-3-Mediated Assembly of IDR-Containing Proteins

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Intrinsically disordered proteins and regions (IDPs/IDRs) represent a broad class of proteins characterized by the absence of a stable, well-defined three-dimensional structure under physiological conditions. Despite this apparent structural ambiguity, they play essential roles in numerous cellular processes and are linked to various diseases. Due to their dynamic nature, IDPs pose a significant challenge for structural characterization, yet understanding their interactions is crucial for drug discovery and the development of targeted therapeutics. To address this challenge, we employed computational approach combining AlphaFold2 (AF2) structural prediction with all-atom molecular dynamics (MD) simulations to investigate heterotetrameric complexes composed of ordered and disordered protein segments. Specifically, we focused on the ordered scaffold protein 14-3-3 and three disease-associated IDR-containing interaction partners—SARS-CoV-2 nucleocapsid (N) protein, pyrin, and p53—all of which are involved in various disease pathways. These partners interact with 14-3-3 via disordered regions, making them more challenging.

For the first time, complete models of these heterotetrameric complexes were modeled. While AlphaFold2 provided initial structures, subsequent refinement using both discrete molecular dynamics (DMD) and classical molecular dynamics (CMD) simulations significantly enhanced the structural accuracy and stability of the complexes. These simulations revealed distinct interaction dynamics between the ordered 14-3-3 protein and its disordered partners. Notably, (i) MD simulations uncovered increased conformational stability and cooperative assembly of IDRs over time. (ii) Trajectory analyses comparing 14-3-3/IDR complexes to IDR proteins alone underscored the critical role of 14-3-3 in stabilizing and organizing disordered regions, thereby influencing their functional states. (iii) A filtering workflow was developed to manage the vast conformational space sampled during long-term simulations, enabling the identification of optimal structural models for downstream in silico applications, such as docking or virtual screening.

Taken together, our findings shed light on how the 14-3-3 scaffolding protein regulates IDRs and stabilizes the flexible regions of their binding partners, which are implicated in viral infection, inflammation, and cancer. By integrating AI-based modeling with molecular dynamics refinements, our approach advances the understanding of the structural and functional roles of IDRs and opens new opportunities for targeting IDR-mediated interactions in drug discovery.



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sciforum-122992: Computational Insights into the pH-Dependent Behavior of Ipilimumab-CTLA-4: A Switchable Antibody

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One of the problems with using therapeutic antibodies is on-target/off-tumor toxicity, which can lead to various adverse effects, including cardiotoxicity, skin rashes, organ inflammation, and more. To minimize the likelihood of this toxicity issue, it is necessary to design a switchable antibody that activates only against cancerous cells and becomes inactive in normal cells. Previous experimental results modified Ipilimumab, an immunotherapy drug used to treat melanoma, into pH-dependent antibodies, which are Ipi95, Ipi105, and Ipi105 variants. This involved mutating six amino acids in their complementary determining region (CDR) of the heavy and light chains to charged amino acids such as Histidine, Glutamate, and Aspartate, which resulted in a better binding affinity to the CTLA-4 antigen in acidic conditions compared to neutral conditions. This work aimed to explain the rationale behind each Ipilimumab variant at the atomic level using computational studies. For each CTLA-4–Ipilimumab variant complex system, five replicas of a 500 ns molecular dynamics simulation were conducted using Amber24. The simulation was conditioned in neutral and acidic microenvironments by distinguishing the protonation states of each charged amino acid. Computational alanine scanning mutagenesis analysis confirmed that all six amino acids are important residues in Ipilimumab–CTLA-4 binding. Each modified Ipilimumab variant exhibits distinct characteristics; however, all performed better in acidic conditions than in neutral conditions, as intended. Further analysis based on surface potential, radius of gyration, number of contacts, and binding affinity calculations is also consistent with the experimental studies. The correlation between experimental and computational binding free energy resulted in an R^2 value of 0.7596, and although the statistical analysis suggests a non-significant difference, the effect size (Cohen's $d = 1.183$, Cliff's $\delta = 0.760$) indicates a potentially meaningful difference between neutral and acidic conditions in the Ipi95 variant. By understanding the effects of mutations on a molecular level, we hope to gain new insights into designing switchable therapeutic antibodies.



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sciforum-123311: Generalised Protein Backmapping with Deep Learning (CNNs): From Coarse-Grained to Atomistic

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Coarse-grained (CG) modeling consolidates multiple atoms into single beads, reducing particle counts and simulation complexity while lowering computational costs. This enables larger systems to be simulated over longer timescales. Coarse-graining converts all-atom (AA) models to CG representations, and backmapping restores the detailed atomic structure.

Transitioning from a high-resolution AA state to a low-resolution CG state is generally more straightforward, as information is lost but not gained.

However, complexity increases transitioning from CG low-resolution to AA high-resolution state. Since information needs to be recovered, backmapping typically requires more steps, including both molecular dynamics (MD) simulations of the AA system, and potentially machine learning (ML)-based training.

Most current ML-based backmapping methods train on data from short AA simulations. These models are often specialised for specific systems or proteins and require large AA MD simulations for training. As a result, developing a functional backmapper for a given system can be time-consuming.

Here, we developed a generalised ML-based model that is trained once and is capable of backmapping any protein. Candidate proteins were selected for training to represent common structures. Twelve proteins covering all CATH classes were simulated for 200 ns since the ML model used only local spatial data. Before training the deep convolutional neural network (CNN) models for protein backmapping, the data was divided into two sets: one for backbone atoms and one for the side-chains. A dynamic min-max normalisation was applied to each fragment and atom individually, ensuring that the global spatial context was encoded while constraining the predicted coordinates.

The model was evaluated using test cases based on MD simulations of several large proteins, e.g., Immunoglobulin E (IgE) antibodies, SARS-CoV-2 spike protein, and capsid protein from dengue virus, which are typical of CG applications. Results showed an average backbone RMSD of 0.4 Å, while the side-chain RMSD was around 1.9 Å. This indicates that the side-chain model requires significant further optimisation to improve overall performance.

Refined model converts CG PDBs to AA PDBs without needing retraining or energy minimisation. Although the current model uses CG Martini-2 force field, future work aims to extend its capabilities to the Martini-3 CG mapping scheme.



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sciforum-122051: GFP-Paint: Using Binding Affinity of GFP-Tagged HSPs to Strainotype alpha-Synuclein Polymorphs

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alpha-Synuclein (α -Syn) aggregation is a hallmark of neurodegenerative diseases such as Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. However, distinguishing between pathological α -Syn conformations remains a significant challenge. This study explores the application of a GFP-Paint technique combined with single molecule fluorescent spectroscopy to visualize heat shock proteins (HSPs) and their binding affinities to various α -Syn fibril polymorphs.

By expressing GFP-tagged HSPs in cell-free lysates, we systematically tested their binding with α -Syn polymorphs under various physiological conditions (high salt, artificial cerebrospinal fluid, metal ion-induced, and saccharide-induced fibril strains). This revealed that HSP40's appear to exhibit the strongest interaction with α -Syn fibrils, whereas other HSP groups showed minimal binding. We also observed binding variation between the fibril polymorphs and individual HSP's tested indicating selective chaperone engagement. Binding specificity and affinity calculations confirmed significantly varied interactions between a panel of HSPs and fibrillar α -Syn strains which may have implications for targeted therapeutic strategies.

These findings highlight a powerful method for distinguishing structural variations in protein interactions at the nanoscale. Given the selective binding of HSPs to distinct α -Syn fibril conformations, this approach could aid in identifying specific α -Syn strains associated with disease progression. By integrating this technique into clinical biomarker pipelines, it may become possible to differentiate between PD and DLB at an early stage, leading to improved diagnostics and therapeutic interventions targeting protein misfolding disorders.



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sciforum-123173: Multiscale molecular modeling of metalation processes in proteins: from recognition to catalysis

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Protein metalation is crucial for the molecular machinery of life. Examples of key metal-dependent processes that living organisms perform include structural stabilization, cofactor embedding, metal trafficking, and mechanisms involved in detoxification. It is also a pillar of biomedicine, as several transition metals have been shown to have a non-innocent impact on neurodegenerative diseases and in biotechnology, where the design of new metalloenzymes holds significant promise for our transition toward greener synthetic practices.

Despite longstanding multidisciplinary efforts by the scientific community to uncover the rules that govern metal–protein interactions, complete atomistic descriptions are often challenging to reach, and answers generally remain partial. Unanswered questions continue to spread over the field. Long-range weak interactions, folding processes, and first and second-coordination sphere electronic effects undoubtedly contribute to recognition. However, how they balance to dictate metal specificity, define the most efficient binding sites, or control the diffusion pathways of metal remain questions we are only beginning to unravel.

Computational tools—particularly molecular modeling—increasingly contribute to our understanding of life's mechanisms. However, modeling how metal moieties interact with proteins remains one of the most challenging tasks. In this talk, I will introduce our latest contributions in the field, including new software development and multiscale modeling studies on metal–proteins interactions.



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sciforum-122040: Sub-ångström resolution structure of an iron-sulfur protein resolved using serial femtosecond crystallography

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To understand the chemical properties of metalloproteins and their dynamics it is essential to obtain fine structural information at resolutions higher than 1.2 Å, and most importantly, using low to radiation-damage free models, thereby enabling reliable atomic refinement with high confidence. Here we applied serial femtosecond crystallography at ambient temperature (rt-SFX) using ultra-short X-ray free electron laser pulses at megahertz repetition rate to obtain ultra-high-resolution structure (0.95-0.984 Å) of a recombinant high-potential iron sulfur protein (HiPIP), a model metalloprotein with a single Fe₄S₄ cluster [1], in its reduced form. HiPIP is a small bacterial photosynthetic protein that mediates the photoinduced electron transfer between cytochrome bc₁ and the light-harvesting reaction center (LH1-RC), enabling the re-reduction of the photo-oxidized LH1-RC in bacterial photosynthesis [2]. Our sub-ångström resolution structure allowed us to unambiguously resolve the Fe₄S₄ cluster and the hydrogen bonding network with nearby residues. Additionally, we resolved a cryogenic synchrotron structure of a reduced HiPIP at a resolution of ~0.94 Å and compared it with the rt-SFX structure. The results reveal key differences between these structures particularly at the cluster site. We found that most of the intra-atomic distances of the Fe₄S₄ cluster are shortened by 0.03 to 0.1 Å in comparison with the cryogenic structure, indicating that the rt-SFX model is less affected by radiation-damage. Secondly, by comparing our structure with a neutron diffraction data of the active state [3], we also observed key differences in the amide protons of key residues within the cluster vicinity which may have an impact on the understanding of HiPIP electron transfer mechanism in bacterial photosynthesis. Finally, we resolved the rt-SFX structure of the oxidized (active) HiPIP at a resolution of 1.35 Å, which enabled us to discuss key differences in the redox tuning of HiPIP. We will highlight the results of the rt-SFX models and their comparison with that of the cryogenic models.

References

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- [3] Hanazono, Y. et al. (2022). *Sci. Adv.* **8**, eabn2276.



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sciforum-136546: Caffeine as an Inhibitor of Amyloid- β Dimerization: A 3D-RISM and Molecular Dynamics Approach

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Alzheimer's disease is a detrimental neurological disorder caused by the formation of amyloid fibrils due to the aggregation of amyloid- β peptide. The primary therapeutic approaches for treating Alzheimer's disease are targeted to prevent this amyloid fibril formation using potential inhibitor molecules. The discovery of such inhibitor molecules poses a formidable challenge to the design of anti-amyloid drugs. This study investigates the effect of caffeine on dimer formation of the full-length amyloid- β using a combined approach of all-atom, explicit water molecular dynamics simulations and the three-dimensional reference interaction site model theory. The change in the hydration free energy of amyloid- β dimer, with and without the inhibitor molecules, is calculated with respect to the monomeric amyloid- β , where the hydration free energy is decomposed into energetic and entropic components, respectively. Dimerization is accompanied by a positive change in the partial molar volume. Dimer formation is spontaneous, which implies a decrease in the hydration free energy. However, a reverse trend is observed for the dimer with inhibitor molecules. It is observed that the negatively charged residues primarily contribute for the formation of the amyloid- β dimer. A residue-wise decomposition reveals that hydration/dehydration of the side-chain atoms of the charged amino acid residues primarily contribute to dimerization.



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sciforum-126016: Computational studies for structural features of tetrameric amyloid β_{1-42} peptides with D-Asp1, 7, and 23 residues.

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Although the proteins of existing living organisms are mainly composed of L-amino acids, D-amino acids are occasionally detected in some proteins. Amyloid β ($A\beta$) has three aspartic acid residues, and D-Asp has been detected in $A\beta$ found in senile plaques in the brains of patients with Alzheimer's dementia. These D-Asp residues have been suggested to be involved in the β -sheet formations and aggregation of $A\beta$, but their effects on the three-dimensional structure of $A\beta$ remain unclear at the molecular and atomic levels. In particular, it has not been clarified experimentally or computationally for the structural features of $A\beta$ in which all Asp residues are stereoinverted. Therefore, in this study, we estimate the three-dimensional (3D) structure of artificial mutant of $A\beta_{1-42}$, in which all Asp residues are converted to D-Asp, using molecular dynamics simulations. In this study, we investigated the 3D structure of $A\beta_{1-42}$ tetramers in implicit and explicit solvent environments. Previous experiments have suggested that stereoinversions of Asp7 and 23 play important roles in β -sheet formations of $A\beta$, while Asp1 stereoinversion is known to counteract their effects. In the computational results for the mutant $A\beta_{1-42}$, in which all Asps were stereoinverted, the effect of stereoinversions of Asp7 and 23 on β -sheet formation was greater than the inhibition by D-Asp1, and the more β -sheet structures were observed in the mutant than in the wild type.



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sciforum-125806: Decomposed matricellular proteins reflect the immune-protease activities of COVID-19

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Introduction

Circulating matricellular proteins, osteopontin (OPN) and galectin-9 (Gal-9), are elevated in COVID-19. Gal-9 structurally consists of two homologous carbohydrate-recognition domains (NCRD and CCRD) linked by a linker peptide highly susceptible to proteolysis. OPN is well known to be cleaved by various proteases such as MMPs and caspase-8/3, along with inflammatory responses. This study aims to clarify whether the cleaved forms of Gal-9 and OPN could reflect the activation of proteases in COVID-19.

Methods

COVID-19-infected patients were divided into 23 with mild clinical symptoms (CV) and 25 associated with pneumonia (CP). The following ELISAs measured plasma levels of OPN and Gal-9. Full-length Gal9 (FL-Gal9) and the sum of FL-Gal9 and NCRD with attached truncated linker peptide were determined using FL-Gal9 and Tr-Gal9 ELISA, respectively, which we have previously developed. N-cleaved-Gal9 levels were calculated by subtracting FL-Gal9 levels from Tr-Gal9 levels. FL-OPN levels were determined using an ELISA kit (JP27158, IBL, Gunma, Japan). The total of FL and cleaved forms of OPN (Ud-OPN) levels were determined using the Human OPN ELISA kit (R&D Systems). Moreover, plasma MMP-9 levels were measured using an MMP assay kit (Millipore, Burlington). Soluble interleukin-2 receptor (sIL-2R) was also measured.

Results

Receiver operating curve (ROC) analysis showed that N-cleaved-Gal9 and Ud-OPN levels discriminated CV from CP with AUC of 0.9076 and 0.8207, respectively. Spearman's correlation analysis showed that positive correlations of N-cleaved-Gal9 levels with sIL-2R ($r = 0.789$) and MMP-9 ($r = 0.519$) levels in CP. Similarly, positive correlation of Ud-OPN levels with sIL-2R ($r = 0.792$) and MMP-9 ($r = 0.381$) levels was observed in CP ($r = 0.792$). In addition, sIL-2R levels were positively correlated with MMP-9 levels ($r = 0.441$) in CP.

Conclusion

The cleaved forms of Gal-9 and OPN help assess the severity of COVID-19 pneumonia. The cleavage and release of sIL-2R are known to be mediated by MMP-9 in inflamed lung tissues. Our correlation results add new knowledge that MMP-9 activity could be involved in Gal-9 and OPN proteolysis along with sIL-2R release in COVID-19 with lung inflammation, suggesting that decomposed Gal-9 and OPN could reflect immune-protease activities in COVID-19.



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sciforum-121362: Design of Ig-like binders targeting α -synuclein fibril for mitigating its pathology

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the accumulation and transneuronal propagation of pathological α -synuclein (α -syn) fibrils. These aggregates not only disrupt neuronal homeostasis but also trigger chronic neuroinflammation, contributing to widespread neurodegeneration. Recent studies have identified several receptors that bind to the C-terminal region of α -syn fibrils, mediating its pathology. In this study, we discovered that two isolated immunoglobulin-like (Ig-like) domains derived from α -syn receptors, specifically the D1 domain of lymphocyte-activation gene 3 (L3D1) and the V domain of advanced glycation end-products (vRAGE), effectively block cell surface binding, prevent neuronal propagation of α -syn fibrils, and reduce microglial inflammatory responses. Building on this finding, we identified two new Ig-like binders, the D1 domain of the cluster of differentiation 4 receptor (CD4 D1) and the D1 domain of the chimeric antigen receptor (CAR D1), which bind the C-terminal of α -syn fibrils with high affinity and alleviate its neuropathology. Furthermore, using structure-based design, we developed a mutant version of CAR D1 (CAR D1_Mut) with enhanced binding affinity to α -syn fibrils, thereby increasing its inhibitory activity against α -syn fibril pathology. Our work presents new strategies for designing Ig-like binders that specifically capture the C-terminal region of α -syn fibrils to mitigate its neuronal propagation and inflammation, offering a promising therapeutic approach to slow PD progression.



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sciforum-121473: Development of an α -synuclein PET tracer for clinical diagnosis of Parkinson's diseases

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In a range of neurodegenerative diseases, the accumulation of misfolded protein fibrillar aggregates is a defining pathological hallmark and serves as a critical diagnostic biomarker. These aggregates include tau and amyloid- β (A β) in Alzheimer's disease, TDP-43 in frontotemporal dementia, and α -synuclein (α -syn) in Parkinson's disease (PD). Among these, α -syn fibrils are of particular interest due to their central role in the formation of Lewy bodies and their close association with disease onset and progression in PD. Targeting such amyloid fibrils is therefore essential for both understanding disease mechanisms and developing accurate diagnostic and therapeutic approaches.

I will highlight our recent progress in elucidating how small-molecule ligands interact with amyloid fibrils—an interaction mode that is fundamentally different from classical ligand–protein binding. Unlike globular proteins, amyloid fibrils present repetitive, solvent-exposed β -sheet interfaces that offer unique binding sites. Through a combination of biochemical, structural, and computational studies, we have gained insights into these distinct binding mechanisms, which in turn guided the rational design of a novel PET imaging tracer.

This PET tracer selectively binds to pathological α -syn fibrils with high affinity and specificity. We have validated its performance across multiple experimental platforms, including in vitro binding assays, cellular models, and several animal models of PD, including mice, rats, and non-human primates (marmosets). The successful development of this α -syn-targeted PET tracer represents a significant advancement in the field, providing a powerful tool for the early and accurate diagnosis of Parkinson's disease and potentially for patient stratification and monitoring therapeutic responses in clinical settings.



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sciforum-112930: Discovery of Antitrypanosomal Gossypol Derivatives Targeting the Glucose Kinases: Insights from Biochemical, Molecular Docking, and In Vitro Biological Methods

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Chagas' disease and human African trypanosomiasis are two of the world's neglected tropical diseases caused by the pathogenic protozoan parasites *Trypanosoma cruzi* and *Trypanosoma brucei*, respectively. There are approximately 130 million people at risk of acquiring these diseases, which principally spans the regions of Central and South America, as well as Sub-Saharan Africa. In a high-throughput screening study previously conducted by our laboratory, there were three derivatives of the natural product ($\pm$)-gossypol that were identified to inhibit the potential drug-target, *T. cruzi* glucokinase (TcGlcK), and the compounds also exhibited good antichagasic activity with respect to benznidazole (a first-line therapeutic). To expand on this previous research, a commercially available library of 88 synthetic gossypol derivatives was used for the screening of TcGlcK in an explorative hit-expansion study. Several derivatives were identified through biochemical assays to be strong inhibitors of TcGlcK. The same compounds also revealed strong antitrypanosomal activity against *T. cruzi* and *T. brucei*, through in vitro biological activity assays. Cytotoxicity was also examined for the compounds of interest against murine NIH-3T3 fibroblasts showing a therapeutic window. The trypanosomatid parasites have a unique set of two glucose kinases, such as hexokinase and glucokinase for *T. cruzi* and two isoenzymes of hexokinase for *T. brucei*. Accordingly, a computational molecular docking assessment was performed on the 3-dimensional structures (either derived from X-ray crystal structures or AlphaFold computed structure models) with the leading gossypol derivative hits. The in silico molecular docking analyses revealed strong binding in the D-glucose binding sites of the different glucose kinases. We plan to present the specific details of these predictive interactions along with the biochemically determined mode of inhibition for each inhibitor assessed.



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sciforum-123117: Exploring ATP Non-Specific Behaviour: How Forcefield Choice Shapes Molecular Dynamics Outcomes

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Beyond its well-known role as a universal energy currency, ATP has recently been recognized for its ability to stabilize proteins and prevent aggregation, even at low physiological concentrations. However, the exact mechanism by which ATP exerts these stabilizing effects remains unresolved. Initial studies proposed that ATP acts as a biological hydrotrope, enhancing protein solubility through non-specific interactions. Subsequent investigations have suggested additional or alternative mechanisms, including specific ion effects described by the Hofmeister series and, more recently, the formation of large ATP clusters on protein surfaces, as demonstrated through molecular dynamics (MD) simulations. NMR experiments have also revealed that ATP preferentially binds to arginine residues on protein surfaces, potentially modulating protein phase behaviour and liquid-liquid phase separation (LLPS). To unravel the mechanism by which ATP modulates protein interactions and assembly, it is essential to accurately characterize its behaviour in aqueous environments. MD simulations offer valuable insight into such molecular phenomena; however, challenges persist due to the tendency of ATP to form overly stable self-interactions in simulations, leading to unphysical aggregation and precipitation. These artifacts complicate efforts to interpret ATP's physiological role using conventional force fields. In this study, we employ refined molecular dynamics parameters, such as modern water models, charge scaling and repulsive terms for the aromatic ring that better reproduce the experimental diffusional and kinetic properties of ATP in solution. We investigate ATP's interaction with lysozyme, a model globular protein, focusing on ATP cluster formation and preferential binding to arginine residues. Our results reveal how ATP-ATP interactions, charge scaling and water models can vary the ATP-Protein interactions, and which parameters are best to reproduce experimental non-specific binding of ATP upon lysozyme.



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sciforum-123244: FLOGEN New Sustainability Framework and the Role of Molecular Sciences and Drug Development

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The FLOGEN sustainability framework, developed by the author in 2015, clarified the confusion that existed in the definition of sustainability by making a clear distinction between criteria, actors and objectives of sustainable development. Since its development, due to its unified, universal and all-inclusive nature, it has been applied in many fields of science and technology and has been presented as an opening plenary lecture at the United Nations Meeting in Geneva and turned into law by various municipalities, which made it the first time ever that a scientific concept is turned into law.

During this conference, this unique framework of sustainability will first be presented as well as its 3 pillars which are: (1) science & technology (2) governance & management, and (3) education & civil society. Secondly, it will also analyze the many applications of this framework in the field of molecular sciences and how fundamental knowledge of protein structure and function analysis can be used to develop sustainable drugs for the benefit of humanity. Finally, it will be highlighted and analyzed the progress and difficulties that arise as well as opportunities in the field of molecular sciences and AI-assisted applications, in the context of this unique framework, as well as the essential and irreplaceable role of science and technology.



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sciforum-123146: From multifunctionality to polypathogenicity with intrinsic disorder

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Intrinsically disordered proteins (IDPs) lack stable tertiary and/or secondary structure under physiological conditions *in vitro*. IDPs are characterized by an astonishing multi-level spatiotemporal heterogeneity, with their mosaic structure representing a complex combination of foldons, inducible foldons, morphing inducible foldons, non-foldons, semi-foldons, and unfoldons.

IDPs are highly abundant in nature and have functional repertoire that is very broad and complements functions of ordered proteins. Often, IDPs are involved in regulation, signaling and control pathways, commonly acting as hubs in protein-protein interaction networks. Intrinsic disorder is an important constituent of the proteoform concept, representing one of the important means of functional diversification of the proteinaceous products of a gene. Functions of IDPs may arise from specific disordered forms, from inter-conversion of disordered forms, or from order $\leftrightarrow$ disorder transitions. The choice between these conformations is determined by the peculiarities of the protein environment, and many IDPs possess an exceptional ability to differently fold in a template-dependent manner. As a result, many IDPs are capable of conducting multiple functions, with such multifunctionality being linked to their spatiotemporal heterogeneity. Therefore, a correlation between protein structure and function represents a “protein structure–function continuum”, where a given protein exists as a dynamic conformational ensemble containing multiple proteoforms characterized by diverse structural features and miscellaneous functions.

IDPs are tightly controlled in the norm by various genetic and non-genetic mechanisms. Alteration in regulation of this disordered regulators are often detrimental to a cell, and many IDPs are associated with a variety of human diseases, such as cancer, cardiovascular disease, amyloidoses, neurodegenerative diseases, diabetes and others. Furthermore, many IDPs are multipathogenic, being associated with the origination and development of a number of different diseases. Therefore, there is a thought-provoking interconnection between intrinsic disorder, cell signaling, and human diseases, with polypathogenicity of the involved proteins being linked to their structural plasticity and multifunctionality



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sciforum-119637: Influence of environmental changes on the conformational and amyloidogenic landscapes of an IDP: a combined biophysical and molecular dynamics investigation

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In the past few years, the double PHD fingers 3 (DPF3) protein isoforms (DPF3b and DPF3a) have been identified as new amyloidogenic intrinsically disordered proteins (IDPs). Although such discovery is coherent and promising in the light of their involvement in proteinopathies, their amyloidogenic pathway remains largely unexplored. As environmental variations in pH and ionic strength are relevant to DPF3 pathophysiological landscape, we therefore enquired the effect of these physicochemical parameters on the protein structural and prone-to-aggregation properties, by focusing on the more disordered DPF3a isoform. In the present study, we exploited *in vitro* and *in silico* strategies by combining spectroscopy, microscopy, and all-atom molecular dynamics methods. Very good consistency and complementary information were found between the experiments and the simulations. Acidification unequivocally abrogated DPF3a fibrillation upon maintaining the protein in highly hydrated and expanded polyelectrolyte-like conformers due to extensive repulsion between positively charged regions. In contrast, alkaline pH delayed the aggregation process due to loss in intramolecular contacts and chain decompaction, the extent of which was partly reduced thanks to the compensation of negative charge by arginine side chains. Through screening attractive electrostatic interactions, high ionic strength conditions (300 and 500 mM NaCl) shifted the conformational ensemble towards more swollen, heterogeneous, and less H-bonded structures, which were responsible for slowing down the conversion into β -sheeted species and restricting the fibril elongation. For defining the self-assembly pathway of DPF3a, we unveiled that the protein amyloidogenicity intimately communicates with its conformational landscape, which is particularly sensitive to modification of its physicochemical environment, therefore acting as a context-dependent polyampholyte IDP. As such, understanding how to modulate DPF3a conformational ensemble will help designing novel protein-specific strategies for targeting neurodegeneration.



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sciforum-122838: Interaction of new mixed-valence Polyoxovanadate clusters with proteins

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Polyoxidovanadates (POVs) constitute a sub-class of polyoxidometalates (POMs) with intriguing physico-chemical, electronic, catalytic, magnetic and, particularly, medical properties. The mechanisms at the basis of POVs biological activity are not completely clear. However, there is no doubt that their binding to proteins plays a crucial role. Recently, high-resolution crystal structures of lysozyme and H-chain human ferritin in the presence of the potential drug $[V^{IV}O(acetylacetonato)_2]$ under different experimental conditions have been solved. The analysis of the electron density maps showed that $[V^{IV}O(acac)_2]$ undergoes degradation, ligands loss and partial oxidation of V^{IV} to V^V producing unexpected mixed-valence polyoxidovanadates with composition $[V_{20}O_{54}(NO_3)]^{n-}$, $[V_{15}O_{36}(OH_2)]^{5-}$, $[V_{15}O_{33}(OH_2)]^+$ and $[V_{20}O_{51}(OH_2)]^{n-}$, which bind covalently and non-covalently to the proteins surface. The partial oxidation of V^{IV} to V^V and the formation of mixed-valence species was also confirmed by EPR spectroscopy. The results of structural analysis suggest that: i) POVs of various dimensions can be formed starting from the same vanadium compound and can interact with the protein; ii) the interaction with the protein can stabilize the structure of unexpected and unprecedented POVs, and iii) protein crystals can be used to trap POVs with unusual structures. Moreover, one protein can interact with more than one POV with the sites exposed on the protein surface, favoring their transport in biological fluids. Finally, our data could be of interest to better interpret and rationalize the in vivo data obtained using POVs.



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sciforum-123195: Lessons about Protein Folding, Binding, and Intrinsic Disorder from the Study of Archetypal Protein Folds

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To operate as biological nanomachines proteins must fold into complex 3D structures, bind selectively to partners, and undergo conformational changes on cue. The native functional structures and the rates of interconversion between conformational states are all encoded in the protein's amino acid sequence, but the physical aspects of this code have proven difficult to crack, in terms of both prediction and understanding the molecular mechanisms at play.

Earlier work on single-domain proteins reported a commonality of slow rates and simple behavior, which suggested an all-or-none process. Without a first-principles pre-exponential factor for protein (un)folding dynamics, the rates could only be interpreted in relative terms, such as mutational effects. Therefore, neither the height of folding barriers nor their entropic contributions were known. Because such rates were also way too slow for direct atomistic simulations, computational methods also focused on investigating the effects of mutation. However, such mutational effects proved to be strikingly homogeneous with all experimental data clustering at $\sim 1/3$ of the free energy perturbation recovered on folding and $\sim 2/3$ on unfolding. Ultrafast kinetic methods changed the scene by allowing measuring the timescales of elementary (un)folding motions, which set the pre-exponential factor for protein dynamics at ~ 1 microsecond. In parallel, we and others set out to investigate the simplest possible protein structures capable of autonomous folding, which we defined as archetypal folds. The rationale was to recapitulate the hierarchical organization of protein structure, starting from the bottom. The study of fold archetypes opened new research avenues in protein (un)folding, and established unanticipated connections with the folding upon binding of intrinsically disordered proteins.

In my talk I will summarize our work on the kinetic, thermodynamic, mechanistic, single-molecule, and functional analysis of fold archetypes and their intimate connection with intrinsic disorder and functional conformational plasticity. I will finish by summarizing some of our recent findings about exciting biological and technological applications of the gradual conformational transitions of fold archetypes.



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sciforum-116886: Metallodrugs Encapsulation Within Ferritin Nanocages

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The encapsulation of anticancer metal-based drugs within a nanocarrier represents a valuable strategy to improve the efficacy and selectivity of these compounds towards cancer cells [1]. Different nanomaterials can be used to this scope [2]. Among them, ferritin (Ft), a 24-mer protein with a peculiar spherical structure, seems promising thanks to its stability, high loading capacity, biocompatibility and cell specificity due to the capacity to bind to receptors overexpressed on cancer cells surface, such as TfR-1 (transferrin receptor 1) and Scara5.

Based on these premises, my research group has been involved in the preparation of several ferritin-metallodrug nanocomposites [3], mainly containing platinum-, ruthenium- and gold-based anticancer agents. The drug-loaded Ft nanocages were characterized from a structural point of view, by means of X-ray crystallography, to identify and to define the type of a possible interaction between the drug and the protein, and from a biological point of view, evaluating their cytotoxicity and searching for the pathways activated by the metallodrug when free and when encapsulated within Ft. ICP (inductively coupled plasma) mass spectrometry helped in the quantification of drug loading. Altogether, our data showed interesting results: i) the overall cage structure is unaltered upon encapsulation, preserving the features of the native protein; ii) a significant amount of metallodrug is trapped in the bulk and may represent the biological active species; iii) the drug-loaded nanocarriers show moderate selectivity toward cancer cells. All these data prove the potential of ferritin as a suitable drug delivery system



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sciforum-119403: Molecular Dynamics of RNA-Guided Endonucleases: Comparative Insights into Cas12 and Eukaryotic Fanzor Systems

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CRISPR and OMEGA systems have revolutionized gene editing. Although several studies have examined individual effectors such as Cas9, Cas12, and Fanzors, a comparative analysis of their structural dynamics and regulatory mechanisms at all-atom resolution is lacking. This study fills this gap by analyzing and comparing nucleic acid–protein allosteric interaction networks for representative members of both families. These include Cas12f and Cas12j from prokaryotes and the eukaryotic Fanzor proteins SpuFz1, GtFz1, PpFz1, and ApmFz2. We conducted molecular dynamics (MD) simulations at the microsecond scale and applied graph theory-based network analysis to identify dynamic communication pathways between the guide RNA, target DNA, and protein domains. Our analysis revealed conserved patterns of allosteric communication in Cas12 and Fz1-Fz2 effectors. Specifically, nucleotides proximal to the PAM or TAM motifs communicate exclusively with residues in the recognition lobes. Nucleotides in the middle of the R-loop exhibit preferential dynamic coupling with residues in the catalytic lobes. In contrast, distal nucleotides in the R-loop demonstrate minimal domain-level communication. Additionally, the nucleotide just before the R-loop formation in the guide RNA engages in bifurcated allosteric signaling, displaying strong coupling with a catalytic domain residue and weaker yet reproducible interaction with a residue in the recognition domain for all systems. The conservation of this feature across the Cas and Fanzor systems suggests a common regulatory checkpoint involving correlated protein communication with the R-loop. This indicates evolutionary consistency from prokaryotes to eukaryotes. This uncharacterized allosteric layer may play a regulatory role in modulating enzymatic activation. It offers critical mechanistic insight that could inform the design of next-generation CRISPR and OMEGA effectors with enhanced precision, tunability, and reduced off-target activity.



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sciforum-126292: Multiscale Simulations and Profiling of Human Thymidine Phosphorylase Mutations: Insights into Structural, Dynamics, and Functional Impacts in Mitochondrial Neurogastrointestinal Encephalopathy

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Introduction

Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE) is a rare, autosomal recessive metabolic disorder that primarily affects the gastrointestinal and nervous systems, with an estimated prevalence of 1-9 per million individuals worldwide. It is caused by mutations in the *TYMP* gene, leading to the loss of human thymidine phosphorylase (HTP) activity and subsequent mitochondrial dysfunction. Despite its well-characterized biochemical basis, the molecular mechanisms by which MNGIE-associated mutations alter HTP's structural stability, dynamics, and substrate (thymidine) binding remain unclear.

Methods

In this study, we employed a multiscale computational approach, integrating AlphaFold2-based structural modeling, all-atom and coarse-grained molecular dynamics (MD) simulations, protein-ligand (HTP-thymidine) docking, HTP-thymidine complex MD simulations, binding free energy landscape analysis, and systematic mutational profiling, to investigate the mechanistic impact of key MNGIE-associated mutations (R44Q, G145R, G153S, K222S, and E289A) on HTP function.

Results

Analyses of long-timescale multiscale simulations (comprising 9 μ s coarse-grained, 1.2 μ s all-atom apo HTP, and 1.2 μ s HTP-thymidine complex MD simulations) and physicochemical properties revealed that while wild-type HTP maintains structural integrity and strong thymidine-binding affinity, MNGIE-linked mutations induced substantial destabilization, increased flexibility, and reduced enzymatic efficiency. Free energy landscape analysis highlighted a shift toward less stable conformational states in mutant HTPs, further supporting their functional impairment. MD-based MM/GBSA analyses revealed diminished binding affinities for thymidine in the mutants, particularly G145R and E289A. Mutational profiling using the Residue Scan protocol implemented in Molecular Operating Environment (MOE) corroborated these findings, with R44Q, G145R, and K222S showing significant destabilization and loss of thymidine binding. Hydrogen bonding analysis and residue decomposition pinpoint disrupted interactions involving key active site residues (e.g., His116, Arg202, Ser217), contributing to impaired substrate recognition. Additionally, the G145R mutation introduces steric hindrance at the active site, preventing thymidine binding and causing off-site interactions.

Conclusion

These findings not only provide fundamental insights into the physicochemical and dynamic alterations underlying HTP dysfunction in MNGIE but also establish a computational framework for guiding future experimental studies and the rational design of therapeutic interventions aimed at restoring HTP function.



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sciforum-127658: New nanomaterials based on bio-active peptides: structural studies and molecular dynamics

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A combined approach of structural studies and dynamic simulations was used to characterize new nanostructures decorated with bioactive peptides for biotechnological applications. A common strategy is the use of natural scaffolds like ferritins (Fts), ubiquitous proteins that self-assemble to form hollow nanocages. Besides the unique architecture, the H-chain of Ft binds the human transferrin receptor, over-expressed on cancer cells surface. Consequently, human Ft nanocages can be used for the selective delivery of anticancer drugs and, in addition, can be functionalized with different peptides bearing diverse activities. This project aims to functionalize a recombinant human Ft, formed by only H-chains (hHfT), engineering the N- or the C- terminus, with a functional peptide, belonging to the marine diatom's silaffin- and sylacidin, named R5. It has been reported that this small peptide induces silica formation in the presence of phosphate and silicic acid in vivo and in vitro. To characterize the chimeric hHfT from a structural point of view, we coupled theoretical and experimental methods. Molecular dynamics (MD) simulations have been used to examine the spatial orientation of the R5 moiety, whether it is displayed on the outer surface, trapped within the nanocage, or directly interacting with the nanocage. These analyses have allowed us to verify that R5 is localized on the external surface of ferritin. This result provides valuable information regarding its accessibility to produce sea inspired structured hybrid biomaterials. Based on the simulation results, the functionalized ferritin was expressed, purified and characterized in its structural features. Native PAGE and TEM analyses confirmed MD simulations showing that hHfT nanocages correctly expose R5 on their external surface. Finally, SEM data offer structural information on the conformation of the produced ferritin-based silica-nanocomposites. To the best of our knowledge, this is the first time that a peptide from marine diatoms is functionalized with a human H-chain ferritin for silica-based nanocomposite formation. These results provide the basis for future functional characterizations to evaluate the potential of this system as a scaffold for the production of different protein-based biomaterials.



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sciforum-122711: Protein metalation by vanadium-based drugs

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Protein metalation, the process associated with the interaction between metal ions/metal complexes and proteins, has a major role in biology: it is involved in the correct folding and function of metalloproteins; it affects the absorption, transportation and storage of metallodrugs within the body, and it has a major role in the design and development of artificial metalloenzymes and protein-based metallodrug delivery systems (1-3). X-ray diffraction experiments are routinely used for the determination of structures of adducts formed upon reaction of metal compounds with proteins (4-7). In my group we recently solved the X-ray structures of several adducts formed upon reaction of V-based drugs and proteins (8-15). Results of these structural analyses will be presented.

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sciforum-121470: Structural polymorphism of α -synuclein amyloid fibrils in Parkinson's disease

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder after Alzheimer's disease, affecting millions worldwide. A key pathological hallmark of PD is the formation of Lewy bodies—intracellular inclusions composed primarily of amyloid fibrils formed by the misfolding and aggregation of the protein α -synuclein (α -syn). Increasing evidence suggests that the aggregation, accumulation, and propagation of α -syn pathological fibrils across neurons are intimately involved in the initiation and progression of PD.

In our research, we focus on unraveling the molecular mechanisms underlying α -syn pathology and translating these findings into potential diagnostic and therapeutic strategies. Specifically, our work centers on two major aspects:

(1) **Structural polymorphism of α -syn amyloid fibrils:** We discovered that α -syn can adopt multiple distinct fibrillar conformations—referred to as amyloid polymorphs—under different physiological and pathological conditions. These structural variants exhibit varied biological activities, including differences in cell toxicity, propagation potential, and immune responses, suggesting that structural heterogeneity is closely linked to disease phenotypes and progression.

(2) **Inhibitor and tracer development:** We designed and screened small molecules that selectively bind to pathological α -syn aggregates, either blocking their formation and spread or enabling their in vivo detection through PET imaging. These molecular tools not only offer promising leads for therapeutic intervention but also lay the groundwork for early and specific diagnosis of PD.

Together, our studies provide new mechanistic insights into α -syn-driven pathogenesis and open new avenues for the development of targeted diagnostics and treatments for Parkinson's disease.



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sciforum-123316: Structure-Guided Analysis and Functional Characterization of a Plant-Derived Coagulant Protein for Synthetic Dye Bioremediation

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Introduction

Synthetic dyes are persistent environmental pollutants with mutagenic and carcinogenic potential, commonly found in industrial effluents. Traditional remediation methods suffer from inefficiency and ecological drawbacks. In this study, we *investigate a coagulant protein from an indigenous plant that exhibits flocculant properties and promising dye-binding activity*. Using a structure-based approach, we explore its potential for sustainable dye degradation and extend our work toward evaluating the toxicity and reuse potential of the treated water, aiming for an end-to-end bioremediation platform.

Methods

The coagulant protein was modelled and validated using Ramachandran plot and structural quality metrics. Binding pocket analysis (SiteMap, Schrödinger Suite 2023-3) and docking studies with 22 synthetic dyes were conducted using AutoDock Vina, followed by MD simulations and MM/GBSA binding energy calculations. The gene was cloned and expressed, with folding and solubility optimization currently in progress to address challenges in recombinant expression. Decolorization efficacy was tested on synthetic dye solutions, and treated water is now undergoing multi-model toxicity screening, including seed germination assays (plant system), acute and developmental toxicity in zebrafish embryos, and cytotoxicity profiling using human cell lines.

Results

Docking studies revealed that 19 dyes exhibited high binding affinities, particularly at pocket 3, with Congo red and Coomassie brilliant blue preferring alternative sites. Bromophenol blue displayed unique dual-site binding outside predicted pockets. MD simulations confirmed the stability of most complexes, and MM/GBSA analysis supported strong binding energetics. Cloning and initial expression trials of the protein have been successfully completed, with optimization of recombinant protein yield and purity currently in progress for functional validation.

Conclusions and Future Directions

This work presents an integrative structure-function study of a plant-derived protein with dual activity in dye sequestration and flocculation. Its ability to reduce dye toxicity in multiple biological systems marks a significant step toward safe and scalable bioremediation. Ongoing protein folding optimization and mechanistic validation will facilitate preclinical development. The *study offers a translational pathway from molecular modelling to environmental application and public health impact*.

*Patent filed: Indian Patent Application No: 202341082868



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sciforum-123207: The Unified Theory of Neurodegeneration Pathogenesis Based on Axon Deamidation

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Until now, neurodegenerative diseases like Alzheimer's and Parkinson's have been studied separately in biochemistry and in therapeutic drug development and no causal link has ever been established between them. This study has developed a Unified Theory, which establishes that the regulation of axon and dendrite-specific 4E-BP2 deamidation rates controls the occurrence and progression of neurodegenerative diseases. This is based on identifying axon-based 4E-BP2 deamidation as a universal denominator for the biochemical processes of deamidation, translational control, oxidative stress and neurodegeneration. This was achieved by conducting a thorough and critical review of 213 scientific publications regarding deamidation, protein synthesis initiation, translational control, neurodegeneration, oxidative stress and by applying my discovery of the fundamental neurobiological mechanism behind neuron-specific 4E-BP2 deamidation to practical applications in medicine. Based on this newly developed Unified Theory and my critical review of the scientific literature, I also designed three biochemical flowsheets of (1) in-vivo deamidation, (2) protein synthesis initiation and translational control, and (3) 4E-BP2 deamidation as a control system of these four biochemical processes. The Unified Theory of neurodegeneration pathogenesis based on axon deamidation developed in this work paves the way to controlling the occurrence and progression of neurodegenerative diseases such as Alzheimer's and Parkinson's through a unique, neuron-specific regulatory system that is 4E-BP2 deamidation, caused by the proteasome-poor environment in neuronal projections, consisting mainly of axons.



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sciforum-121061: Transcription factor clustering shapes chromatin dynamics to regulate enhancer-promoter interactions

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Enhancers regulate gene expression by establishing long-range contacts with promoters, and phase-separated condensates formed by transcription factors (TFs) and cofactors have been implicated in both promoting and hindering these enhancer-promoter (E-P) interactions. However, the molecular mechanisms underlying this dual role remain unclear. Moreover, the inherent differences in the dynamic properties of chromatin fibers (e.g., euchromatin vs. heterochromatin) may lead to distinct E-P contact behaviors under the influences of TFs, and this has yet to be explored. To address these questions, we developed coarse-grained chromatin models that incorporate varying levels of TF phase-separated clustering, a range of E-P genomic distances, and two chromatin dynamic states (stable and dynamic). We found that the highest E-P contact probability corresponds to a moderate level of TF clustering, in agreement with recent experimental findings. We uncovered that TFs exert a dual role by both promoting and hindering E-P interactions, and that enhancing TF clustering capability can reduce or even eliminate the differences associated with varying E-P genomic distances. Consistent with experimental observations, our results showed that the encounter times of E-P pairs are much less dependent on genomic distance than predicted by existing polymer models. Furthermore, we observed that chromatin chains with different dynamic properties exhibit significantly different capacities for enriching TF clustering at E-P loci, which in turn influences E-P contact. Our findings suggest that dynamic chromatin facilitates E-P proximity by enhancing TF clustering and subsequently promotes direct E-P interactions by destabilizing the TF clusters through chain flexibility. This work provides insights into the chromatin chain dynamics in gene expression, highlighting the critical impact of dynamic chromatin structure on genome organization and functional regulation.



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sciforum-123179: Unravelling structure-function relationships in KCTD proteins

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Introduction

KCTDs, proteins containing the potassium (K) Channel Tetramerization Domain, represent an emerging family involved in fundamental physiological and pathological processes, including cancer, neurodevelopmental disorders, and genetic diseases. Although their biological significance is well established, defining the structure-function relationships of these proteins has proven to be a long and arduous journey. Over the years, we have contributed to unveiling the role of some individual members of this family in distinct pathological contexts (1-4). However, despite our efforts, atomic-level structural characterization was achieved only for KCTD1 using X-ray crystallography (5).

Methods

The possibility to predict reliable protein structures from their sequences, guaranteed by recently released computational approaches, has transformed this scenario, allowing the simultaneous elucidation of structure-function relationships across the entire family. Here, insights into structure-function relationships were achieved through integrated structural biology approaches developed by combining experimental and computational methodologies.

Results

Using this strategy, we discovered previously undetected similarities among the family members, which enabled us to generate a new structure-based phylogenetic tree (6). Moreover, we created reliable three-dimensional structures of all family members in their functional oligomeric states (7). Finally, we defined the structural basis of molecular recognition for many of their biological partners (8).

Conclusion

The functional implications of these findings will be illustrated.

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Session 2. Enzymes

sciforum-126095: Engineered-Enzyme Biomass Fuel Cells: Enhancing Bio-electrochemical Efficiency for Sustainable Energy

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The increasing demand for renewable and sustainable energy sources has spurred innovations in biomass fuel cell technologies. This study reports the development of a high-efficiency, oxygen-insensitive biomass fuel cell through enzyme engineering and advanced electrochemical strategies. The anode design utilizes a synergistic enzyme pair: β -glucosidase (BGL) from *Agrobacterium tumefaciens* (BGL5, GH1 family) and FAD-dependent glucose dehydrogenase (FAD-GDH) from *Talaromyces emersonii*. Both enzymes were heterologously expressed in *Escherichia coli* using pET30a and pET32a vectors. Kinetic characterization showed high catalytic efficiencies: BGL5 exhibited a K_m of 0.17 mM and V_{max} of 283 $\mu\text{mol}/\text{min}\cdot\text{mg}$ for pNPG, while FAD-GDH showed a K_m of 412 mM and V_{max} of 7039 $\mu\text{mol}/\text{min}\cdot\text{mg}$ for glucose. A rationally engineered BGL5 mutant (H229S) demonstrated significantly improved glucose tolerance, with an IC_{50} of 1320 mM compared to 94 mM for the wild type.

The electrochemical system was assembled using wild-type BGL in 100 mM cellobiose at pH 7.0, with co-immobilized TeGDH, polyethylenimine-ferrocene (PEI-Fc) and poly(ethylene glycol) diglycidyl ether (PEGDGE) on a graphene electrode. BGL hydrolyzes cellobiose into two molecules of D-glucose, which are subsequently oxidized to gluconolactone by TeGDH, with PEI-Fc facilitating mediated electron transfer between TeGDH and the electrode surface. On the cathode side, horseradish peroxidase (HRP) was covalently immobilized using 1-pyrenebutanoic acid succinimidyl ester (PBSE), enabling π - π stacking on a graphene-coated polyimide electrode. The configuration was further stabilized with 0.5% Nafion, facilitating efficient hydrogen peroxide reduction using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ammonium salt (AzBTS) as a redox mediator.

This biofuel cell configuration achieved a maximum power density of 126 $\mu\text{W}/\text{cm}^2$ with an open-circuit voltage of 0.6 V, —sufficient to power low-energy electronic devices. The system's oxygen insensitivity, high catalytic performance, and improved glucose tolerance make it a significant step toward practical applications of bioelectrochemical energy systems. These findings highlight the potential for enzyme-engineered, graphene-based biomass fuel cells in the future of sustainable energy technologies.



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sciforum-124951: Histone deacetylases 1 and 6 in migraine: potential therapeutic targets?

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Introduction

Although migraine is one of the most common causes of disability worldwide, its mechanisms of pathogenesis are poorly understood. Except for a few rare migraine variants, the roles of genetic and epigenetic mechanisms in migraine pathogenesis remain largely unknown. The aim of this study was to evaluate the expression of histone deacetylases 1 and 6 (HDCA1 and HDCA6) genes, which are enzymes that epigenetically control gene expression, in the blood of migraine patients and healthy controls. Both HDCA1 and HDCA6 may facilitate chromatin relaxation, potentially contributing to increased gene expression. Additionally, HDCA6, through the acetylation of non-histone proteins, is involved in cytoskeletal dynamics and protein quality control.

Methods

This study included 32 low-frequency episodic migraine patients and 28 controls without headaches. Blood samples were collected during the interictal phase of migraine. The expression of the HDCA1 and HDCA6 genes was determined by real-time qPCR, and the levels of HDCA1 and HDCA6 proteins were assessed using the ELISA test.

Results

We observed a decrease in HDCA1 expression in migraine patients compared to controls ($p < 0.01$). No difference was noted for HDCA6. Similar trends were observed for the HDCA1 and HDCA6 proteins. We observed several correlations between HDCA1 expression and migraine characteristics, including migraine intensity, headache frequency, and the mental state of migraine patients.

Conclusion

Therefore, migraine patients may be characterized by lower expression of specific genes regulated by HDCA1, which could be further investigated in future research. Overall, our study suggests that epigenetic control may represent an important factor in migraine pathogenesis and, therefore, could be considered in its therapy, with HDCA1 potentially targeted to enhance its expression. However, bioinformatic, experimental, and clinical studies are necessary to clarify how HDCA1 might serve as a therapeutic target in migraine.

Acknowledgement

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sciforum-123162: LsAA9A Lytic Polysaccharide Monooxygenase: from initial structures to advanced crystallography of a model copper enzyme

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Lytic polysaccharide monooxygenases are a fascinating class of copper enzymes first recognized as important components of the plant cell wall degradation machinery of microbes about 15 years ago. Through a fairly simple active site containing the characteristic His brace motif LPMOs can degrade even recalcitrant crystalline polysaccharides such as cellulose and chitin. I will briefly introduce the main structural characteristics of known LPMOs and LPMO-like families with examples from my own research. In particular, I will focus on interactions with saccharide substrates, based on our favourite model enzyme LsAA9A.

While the initial impetus in LPMO research came from biotechnological applications such as bioethanol production from lignocellulose, recent work has highlighted diverse roles in nature. With the increasing recognition of the role of LPMOs in human, crop plant and fish pathogens, it is interesting to consider the few known inhibitors and the difficulty of specifically inhibiting such an exposed active site, the very structural characteristic that likely facilitates access to crystalline substrates.

The chemistry of the reaction has received much attention and has been the subject of debate, though most groups now agree that the most likely co-substrate is hydrogen peroxide rather than molecular oxygen. My group has taken a very structure centered approach in exploring mechanistic aspects, using advanced X-ray crystallography to elucidate protonation states of key residues and small structural changes along the reaction mechanism, which will be the topic of the latter part of my talk.



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sciforum-126497: Bi-Enzymatic Biocatalytic System of β -Glucosidase (TxGH116)/Glucose Oxidase for a Cellobiose/ H_2O_2 Biofuel Cell

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We report the development of a novel bioanode based on a bi-enzymatic cascade involving β -glucosidase (TxGH116) and glucose oxidase (GOx). A glassy carbon electrode was modified with GOx immobilized in an osmium-based redox polymer. In the electrolyte, cellobiose—serving as the fuel—is hydrolyzed by β -glucosidase into two molecules of β -D-glucose. The resulting glucose is then oxidized by GOx to D-gluconolactone, with electron transfer mediated by the osmium redox polymer. For the GOx-modified electrode, the effects of enzyme loading and the enzyme-to-polymer ratio were also optimized. The catalytic activities of both enzymes were characterized at various temperatures to assess their kinetic performance.

This bioanode was coupled with a biocathode based on horseradish peroxidase (HRP), in which hydrogen peroxide (H_2O_2) is reduced through a mediated electron transfer process. The complete biofuel cell was evaluated using both pure cellobiose and sugarcane leaf-derived hydrolysate. At 40 °C, the system achieved a maximum power density of $33.7 \mu\text{W cm}^{-2}$ and an open-circuit voltage (OCV) of 0.57 V using 30 mM cellobiose in citrate-phosphate buffer (pH 5.5) and 10 mM H_2O_2 in phosphate buffer (pH 6.0). The biofuel cell demonstrated efficient conversion of biomass into electricity. When fueled with hydrolysate prepared from 100 mg of sugarcane leaves using a cellulase complex, the system delivered a comparable power output, confirming its potential for bioenergy applications.



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sciforum-122778: Breaking down of plastic waste via insect enzymes

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Plastic is an essential component in our modern life, playing an important role in various industries, from food packaging to healthcare and technology. Its affordability and durability make it integrated into everyday activities. However, the accumulation of plastic waste has led to a major environmental problem. The improper disposal of plastic waste has led to ocean pollution, threats to wildlife, and microplastic contamination. According to the UNEP, plastic waste is expected to reach 26 billion tons by 2050, with over half ending up in landfills and oceans, causing severe environmental pollution. Incineration, recycling, and landfilling are common solutions for managing plastic waste. However, these approaches are not fully efficient. In the case of incineration and landfilling, contamination of the environment and economic loss happens. Plastic recycling often results in low-value products with detrimental properties. The biological degradation of plastics is considered an alternative approach to managing plastic waste.

The main objective of this research is the identification and characterization of plastic-degrading enzymes from insect origin. Several potential plastic-degrading enzymes were identified via gene mining DNA databases of different insect genomes using previously characterized plastic-degrading enzymes (PETase and MHETase). The DNA sequences of potential candidate genes were custom-synthesized for protein expression using the pET system. Protein induction was performed in *E. coli* BL21 (DE3) using Isopropyl β -D-1-thiogalactopyranoside (IPTG). The enzymatic activity of the purified protein was measured on various long-chain hydrocarbon substrates, with enzyme kinetics assays conducted to determine key parameters such as the Michaelis-Menten constant (K_m) and maximum reaction velocity (V_{max}). These kinetic measurements provided insight into the enzyme's substrate specificity and catalytic efficiency. Additionally, the purified enzyme was incubated with different plastic polymers to evaluate its degradation capacity. Plastic degradation was assessed by examining morphological changes on the plastic surface, quantifying mass loss over time, and identifying degradation products via gas chromatography (GC). Together, these data will offer a comprehensive understanding of the enzyme's catalytic behavior and its potential application in plastic bioremediation.



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sciforum-129173: Chitin deacetylases for the production of sequence-defined chitosan oligosaccharides. Understanding and Engineering specificity

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Chitin is a linear polysaccharide of β -1,4-linked *N*-acetylglucosamine (GlcNAc) monomers, whereas chitosans are highly or fully deacetylated chitin polymers. Depolymerization of chitin results in chitin oligosaccharides (COS) and de-*N*-acetylation of chitin and COS yield chitosans and partially acetylated chito-oligosaccharides (paCOS), respectively. COS and paCOS have gained recent interest due to their biological activities as antifungals in agriculture for crop protection against fungal pathogens, stimulants of plant growth or improving plant abiotic stress tolerance, and as immunostimulants in animals, being target compounds for sustainable and environmentally friendly applications [1]. Their biological activities not only depend on the degree of polymerization (DP) and degree of acetylation (DA) but also on their pattern of acetylation (PA) that defines the distribution of *N*-acetylglucosamine (GlcNAc) and glucosamine (GlcNH₂) units along the oligosaccharide chain. Current chemical production of COS and paCOS (by depolymerization and deacetylation of chitin under harsh acidic and basic conditions) render complex mixtures with random deacetylation patterns. Most of the bioassays are performed with mixtures of products with inconsistent results due to batch-to-batch variations.

We are developing a bottom-up approach in which COS are first produced by engineered *E. coli* strains expressing rhizobial chitin synthases produce COS with defined DP from DP4 to DP7 depending on the enzyme and engineered active site mutants [2][3]. Next, enzymatic deacetylation of COS with chitin deacetylases (CDAs) has become an attractive alternative to access sequence-defined paCOS and a number of CDAs with different regio-selectivities have been identified and characterized in the last decade [4][5].

Chitin deacetylases (CDA) are members of family 4 carbohydrate esterases (CE4) which operate by metal-assisted general acid/base catalysis. We are interested in understanding the structural bases of substrate specificity by CE4 enzymes, their mechanism of action and biological functions, as well as the use of engineered variants as biocatalysts for the production of sequence-defined paCOS. Here we will report on recent developments to expand the toolbox of CDAs as biocatalyst to access a panel of tailored paCOS: *Vibrio cholera* CDA and engineered variants for single regio-specific deacetylation on which the subsite capping model was first proposed [6], *Pochonia chlamydosporia* CDA and *Pestalotiopsis* sp. CDA with a distributive mode of action, and more recently the extension to some peptidoglycan deacetylases that are also active on COS [7].

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sciforum-122779: Computational studies on carbohydrate-active enzymes

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Carbohydrates are fascinating molecules with biological functions that span from energetic and structural roles, to molecular recognition processes in life development and in disease, as well as the modulation of biological and physico-chemical properties of the parental molecule they can be attached to. No less interesting are the different families of enzymes that assemble (glycosyltransferases, GTs) and disassemble (glycosidases, GHs) monosaccharide units to generate the repertoire of glycans that exist.

In this talk, an overview of some of the computational studies performed in our group over the last decade on carbohydrate-active enzymes will be given. We are interested in the molecular-level understanding of enzyme function for biomedical and biotechnological applications. This includes quantum-mechanical molecular-mechanics (QM/MM) calculations of reactions mechanisms, where we contributed to describe the currently accepted front-side attack mechanism for retaining GTs;¹⁻³ also the characterization of ligand pathways (with GPathFinder) that has led to the proposal of a novel processive-like mechanism for an exo-hydrolase (family GH3);^{4,5} and the exploration of the protein's conformational space by enhanced sampling molecular dynamics (i.e. GaMDs) to find catalytic conformations that orchestrate regioselectivity in di C-glycosylation. If time permits, a couple of examples of applications of protein networks analysis in the context of enzyme design will also be commented.⁶

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sciforum-123072: Construction of Rice Bran-Derived Glucosylceramide Liposomes and Their Application in Subcellular Enzyme Activity Analysis

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We successfully developed a liposomal system primarily composed of high-purity ($\geq 95\%$) rice bran-derived glucosylceramide (GlcCer), demonstrating excellent biocompatibility, biodegradability, and suitability for biological applications. This novel plant-based nanocarrier exhibits rapid cellular internalization, providing a sustainable and innovative for real-time detection of enzymatic activities within specific subcellular compartments. Following cellular uptake, the liposomes display targeted migration to lysosome-like structures and endoplasmic reticulum (ER)-associated regions, enabling spatiotemporally controlled substrate delivery to functionally distinct organelles.

To evaluate the intracellular transport potential of the GlcCer-based liposomes, we designed a FRET-based dual-fluorophore system by incorporating a glucosylsphingosine-derived fluorescent probe (GlcSph-BODIPY) into the liposomal membrane and encapsulating Cascade Blue dye within the aqueous core. This configuration formed a fluorescence resonance energy transfer (FRET) pair between the membrane-bound and core-localized fluorophores. Changes in FRET efficiency allowed us to monitor liposomal integrity and degradation kinetics across different subcellular compartments, providing key insights into the carrier's delivery behavior.

To further assess intracellular substrate release and enzymatic conversion, 4-methylumbelliferone- α -glucoside (4MUG α) and 4-methylumbelliferone- β -glucoside (4MUG β) were separately loaded into the liposomes. Real-time confocal laser scanning microscopy revealed efficient substrate hydrolysis within enzymatically active zones, enabling high-resolution visualization of glycosidase activity distribution and dynamics.

GlcCer-liposome platform has proven to be an effective tool for intracellular enzymatic function analysis. It enables organelle-level precision in substrate delivery and significantly advances real-time monitoring technologies for enzymatic processes, overcoming key limitations of traditional dynamic single-cell enzyme analysis.



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sciforum-114006: Effect of dietary phytase on gene expression of myo-inositol transporters in chicks jejunum

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The mode of action of dietary phytase (PHY) is associated with the destruction of phytate and lower phytate esters in the gastrointestinal tract, alleviation of their antinutritional properties, and the release of myo-inositol (MYO). The MYO is an essential constituent of cellular phosphoinositides and is involved in various cellular functions. Free MYO can be actively transported with high efficiency via three co-transport systems, two are sodium dependent (SMIT1 or SLC5A3 and SMIT2 or SLC5A11) and one is proton dependent (HMIT or SLC2A13). The MYO may also influence intestinal alkaline phosphatase (iALP) activity. However, research with different animals including rabbits, rats, broilers and laying hens showed different response not only between but also within species. The aim of this study was to compare the expression of SMIT1, SMIT2 and iALP in the jejunum of broiler chickens fed low phosphorus control diet (C) and C+PHY. The expression of SMIT1 was upregulated in PHY fed birds ($P < 0.05$) but no response ($P > 0.05$) was observed in SMIT2 expression. Birds fed PHY had also upregulated gene expression ($P < 0.05$) of iALP in jejunum. Previous research (Walk et al. 2018) reported an increase in iALP expression in the jejunum of phytase fed birds but not in the SMIT1 and SMIT2 expression. However, the expression of SMIT2 gene was upregulated in the ileum of phytase fed birds. Regardless, it would appear there is an effect of dietary PHY / MYO concentration in the intestinal lumen on the upregulation of transporters in the jejunum of poultry. The upregulated iALP expression in the jejunum of birds fed PHY suggests that the response to SMIT1 and iALP in the jejunum was associated with lower phytate esters and the production of inositol by iALP.



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sciforum-122965: Effect of Skeletal Muscle Stretching on Factors Affecting Collagen Cross-linking

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Introduction

Collagen in skeletal muscles is essential for tension transmission and regeneration. Moderate collagen cross-linking stabilizes the extracellular matrix (ECM), whereas excessive cross-linking leads to fibrosis. Cross-linking is influenced by advanced glycation end products (AGEs) and lysyl oxidase (LOX). AGEs, formed by sugar-protein reactions, readily bind to collagen, whereas LOX promotes cross-linking in response to mechanical stress. Although tissue-specific differences exist between tendons and ligaments, the mechanisms of intramuscular collagen cross-linking and its mechanical regulation remain unclear. This study investigated the effect of mechanical stretching on LOX and AGEs expression and localization in skeletal muscle.

Methods

Male C57BL/6J mice (12 weeks old) were assigned to the control, intermittent stretching (IS), or continuous stretching (CS) groups. The tibialis anterior muscle was stretched for six days: 10 cycles/day of 1-minute stretches with 30-second breaks and 10 minutes of continuous stretching for the IS and CS groups, respectively. The muscles were collected 24 hours post-intervention. The right muscle was frozen for morphological analysis, and the left was preserved for molecular analysis.

Results

Muscle weight was not significantly different between groups. Gene expression analysis showed that *Col1a1* levels were not significantly different in each group. *Lox* expression significantly increased in the CS group compared to the IS group. *Mmp2* significantly reduced in the IS group but not in the CS group. Immunohistochemical staining revealed collagen I in the endomysium and perimysium of all the groups. The localized region of collagen I in the CS group was significantly larger than in the control and IS groups. AGEs were localized in the ECMs without group differences. LOX was present in the cytoplasm, nuclei, and ECM of muscle fibers in all groups. LOX-positive cells in the ECM significantly increased in both IS and CS groups.

Conclusions

Continuous stretching promotes collagen accumulation, whereas intermittent stretching does not. Both stretch types increased the number of LOX-positive cells in the ECM without altering AGEs localization. These findings suggest that collagen accumulation depends on the stretch type and that LOX-mediated cross-link formation can be promoted by mechanical stretching, regardless of the method used.



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sciforum-122691: Engineering hydrogen peroxide tunnel for generating non-natural P450 peroxygenases

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Given prominent physicochemical similarities between H_2O_2 and water, we talk about an emerging strategy for generating/enhancing the peroxygenase activity of cytochrome P450 monooxygenases by engineering their water tunnels to facilitate H_2O_2 access to the heme center buried therein. Specifically, the H_2O_2 -driven activities of several native NAD(P)H-dependent P450 enzymes (e.g., CYP199A4, CYP153A_{M.aq}, P450BM3, CYP154C5, and CYP105D18) increase significantly (up to ~200-fold improved). Molecular dynamics (MD) simulations and crystallographic studies provides mechanistic insights into the enhancement of peroxygenase activities by mutating the residue at the bottleneck of the water tunnel, which may open a second pathway for H_2O_2 to flow to the heme center (in addition to the natural substrate channel). Furthermore, the strategy of tunnel engineering can be improved by the combination with the introduction of an acid-basic catalytic residue or tunnel redesign by the guidance of MD simulations and crystallographic analysis. The generated non-natural P450 peroxygenases showed excellent catalytic efficiency for a variety of monooxygenation reactions, such as the regio- and enantioselective hydroxylation of long-chain fatty acids and steroids, as well as the demethylation of aromatic ethers hydroxylation. This talk highlights a promising, generalizable strategy whereby P450 monooxygenases can be modified to adopt peroxygenase activity through H_2O_2 tunnel engineering, thus broadening the application scope of P450s in synthetic chemistry and synthetic biology.



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sciforum-122634: Finding of phylogenetically, functionally, structurally, and mechanistically new glycoside hydrolase family enzymes acting on β -1,2-glucans

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β -1,2-Glucans are glucose polymers which play important roles such as pathogenesis, symbiosis and osmoregulation. In *Escherichia coli*, β -1,2-glucan chains with branches are synthesized in periplasm and are called osmoregulated periplasmic glucans (OPG). Defection of OPG synthesis results in less mobility, raising resistance to antibiotics and defense system of a silkworm as a host. However, a biosynthetic pathway of OPG has not been identified biochemically. We started with investigation of OpgD and OpgG based on involvement of *opgD* and *opgG* genes in OPG biosynthesis presumed by genetic analysis.

OpgD and OpgG hydrolyzed β -1,2-glucans endolytically to produce β -1,2-glucooligosaccharides. This is the finding of a phylogenetically new family of glycoside hydrolases (GHs) and a new GH family, GH186, was established for this group of proteins. The complex structures of OpgD and OpgG with β -1,2-glucans suggested a unique reaction mechanism that transfer of a proton on the reaction route is mediated by three water molecules. These water molecules are perfectly sequestered from solvent by a loop region in OpgD while this sequestration is incomplete in OpgG, which causes much lower hydrolytic activity of OpgG than that of OpgD. Interestingly, this loop region is quite diversified in amino acid sequence, suggesting functional diversity within GH186.

Xanthomonas species, a phytopathogen for various plant hosts, produces α -1,6-cyclized β -1,2-glucan (C β G α) composed of 16 glucose units, which is essential for pathogenicity to model plants. However, no enzyme synthesizing C β G α s was identified. We found that OpgD from *Xanthomonas campestris* (XccOpgD) is a transglycosylase to produce the C β G α . The binding mode of a linear β -1,2-glucan in a crystal structure of XccOpgD revealed that glucohexadecaose can be produced by transferring β -1,2-linkage to α -1,6-linkage intramolecularly. This is also the first clear structural evidence of anomer-inverting transglycosylase among GH enzymes. Overall, phylogenetically, functionally, structurally and mechanistically new GH enzymes were found from a model bacterium and a phytopathogen.



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sciforum-126331: Formate dehydrogenase for detection of NAD⁺ in biological samples

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Introduction

Nicotinamide adenine dinucleotide (NAD⁺) is a biological molecule of systemic importance which is involved in various metabolic and signaling pathways. There are a lot of information in literature about correlation between NAD⁺ level in cells and age as well as the coenzyme is also involved in signaling of perturbed homeostasis and DNA damage response. We develop an efficient method of NAD⁺ analysis in whole human blood, applicable for better diagnostics of different systemic pathologies. Following the NAD⁺ dynamics in a patient may help assessing the state of critically ill patients and efficiency of therapeutic approaches.

Methods

Gene of NAD⁺-dependent formate dehydrogenase from bacterium was selected to develop biocatalyst for NAD⁺ determination in biological samples. Structure modelling used to select positions for site directed mutagenesis (rational design approach). New efficient approach for amino changes predictions was also developed. All mutants were expressed in *E.coli* cells as active enzymes. The FDHs were purified and characterized in catalytic properties and stability.

Results

We searched FDH genes in genomic data bases and analyzed structure-properties relationships. "Classical" bacterial FDHs with length around 400 aa were choose as started enzymes. Final selected FDH had the high kinetic properties as well as high temperature stability. Different types of rational design approaches used to select positions in protein to improve catalytic properties as well as enzyme stability against different factors. As result K_M values for coenzyme and formate were improved by 5 and 12 times, respectively. Final version of FDH didn't loose activity at least for 6 months at +4 °C.

Conclusions

We created biocatalyst based on bacterial formate dehydrogenase for NAD⁺-determination in biological samples. Final version of engineered FDH combined high catalytic properties and very high stability under storage, high temperature and proteases degradation. Super expression system of FDH will be developed and large scale down stream process of preparation will be created.

The work was done as state scientific assignment "Determination of the coenzyme nicotinamide adenine dinucleotide (NAD⁺) in biological samples to determine the status of the organism" (KBK 386 0908 012C4 73790 611 12) founded from Russian national project "New technologies for preserving health"



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sciforum-114007: Gene expression of jejunal tight junction proteins in chickens fed supplementary myo-inositol

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The gastrointestinal mucosal barrier plays an important role in maintaining gut health, separating the inside of the body from the outside environment. Tight junctions (TJs) are intercellular adhesion complexes that are essential to the barrier function of epithelia and endothelia. The TJs are composed of transmembrane proteins, including claudins, occludin (OCLN), junctional adhesion molecules and cytosolic proteins. Feeding diets high in phytate, a well-recognized gut irritant, to young poultry may lead to disruption of epithelial TJs and gut barrier dysfunction. Recent interest in supplementing poultry diets with higher doses of phytase, sometimes referred to as “super-doses” of phytase, has led to complete phytate dephosphorylation, providing more myo-inositol (MYO) in the small intestine of poultry. The study aimed to compare the expression of OCLN and tight junction protein 1 (TJP1) in the jejunum of broilers chickens fed graded levels of dietary MYO including 0, 1, 2 and 3 g/kg diet. Supplementing diets with MYO tended ($P = 0.01$) to reduce OCLN jejunal expression and there was a linear OCLN reduction with the increase of MYO inclusion ($L = 0.05$). There was no difference in TJP1 expression ($P > 0.05$) although a linear trend ($L = 0.01$) of reducing TJP1 expression in jejunum with MYO dietary inclusion was observed. The results showed that supplementary MYO did not have an impact on the gene expression of jejunal tight junction proteins in the present study. Thus, suggesting that either phytate did not irritate birds gut, or supplementing MYO to diets in this amount was not enough to change the expression of the studied genes. This also coincided with lack ($P > 0.05$) of differences in feed intake, growth and feed efficiency.



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sciforum-125895: Structure-functional main features of the enzymes, expressed by lactobacilli in response to *Klebsiella*

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Introduction

Antibiotic resistance is a serious problem nowadays. It is important to develop new drugs against pathogenic infections. It was revealed that some *Lactobacilli* strains can suppress *Klebsiella* by secreting enzymes of nucleic acids metabolism, metabolic enzymes and enzymes, degrading of bacterial cell walls (Savinova OS et al. (2021) Int. J. Mol. Sci. 2021, 22, 10999). We cloned and characterized the enzymes from each group which are secreted in the greatest quantities: ribonucleoside hydrolase C (RihC), L-lactate dehydrogenase (LLDH), cysteine synthase A (CysK), bifunctional metallophosphatase/5'-nucleotidase (BM), Zn-dependent peptidase (M23).

Methods

The enzymes were obtained by gene cloning and expression in *E.coli* cells. The purification procedure included metal-chelated chromatography and gel filtration. Activity assay was performed by HPLC and by spectrophotometric methods. Thermal stability of the enzymes was studied by differential scanning calorimetry. Structure analysis was made by alignment, AlphaFold modelling and RSA. Antimicrobial activity was studied on **ESKAPE** (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*) + *E. coli* (totally 35 strains)

Results

Genes encoded RihC, LLDH, CysK, BM, M23 were cloned and the enzymes were expressed in *E.coli* cells in active and soluble form. It was revealed, that *Lactobacilli* enzymes are rather stable and active. The k_{cat} for RihC and LLDH were higher than for orthologs from other sources. RihC, CysK and LLDH crystals were obtained and 3D structures were solved. It was found that RihC and LLDH are tetramers, CysK is a dimer. Sequence and structure of RihC, CysK and LLDH are comparable for enzymes from other bacteria. M23 peptidase molecular modeling revealed by that it has catalytic domain and cell-wall binding domain(s), different in comparison with other M23 proteases. RihC and CysK were active against pathogen biofilms, protease M23 showed antibacterial activity by itself and together with some antibiotics.

Conclusions

The enzymes from *Lactobacilli* are unique, active and stable. Study of them can help to understand the mechanism of interaction between normal and pathogenic microbiota and may help to develop new antibacterial drugs.

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sciforum-122604: The human gut microbiota-plant cell wall nexus: Enzyme discovery and applications

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The human gut microbiota is a vast repository of plant cell wall-active enzymes. This lecture will present latest advances from the laboratory regarding the structure-function analysis of carbohydrate-active enzymes and glycan-binding proteins from Polysaccharide Utilization Loci of symbiotic gut bacteria [1]. Potential applications to improve human health will also be discussed.

For example, mixed-linkage $\beta(1,3)/\beta(1,4)$ -glucan (MLG) is abundant in the human diet through the ingestion of cereal grains and is widely associated with healthful effects on metabolism and cholesterol levels. MLG is also a major source of fermentable glucose for the human gut microbiota (HGM). Bacteria from the family Prevotellaceae are highly represented in the HGM of individuals who eat plant-rich diets [2]. We have defined and functionally characterized an exemplar Prevotellaceae MLG polysaccharide utilization locus (MLG-PUL) in the type-strain *Segatella copri* (syn. *Prevotella copri*) DSM 18205 through transcriptomic, biochemical, and structural biological approaches [3]. In particular, structure-function analysis of the cell-surface glycan-binding proteins and glycoside hydrolases of the *S. copri* MLG-PUL revealed the molecular basis for glycan capture and saccharification. Notably, syntenic MLG-PULs from human gut, human oral, and ruminant gut Prevotellaceae are distinguished from their counterparts in Bacteroidaceae by the presence of a $\beta(1,3)$ -specific endo-glucanase from glycoside hydrolase family 5, subfamily 4 (GH5_4) that initiates MLG backbone cleavage. The definition of a family of homologous MLG-PULs in individual species enabled a survey of nearly 2000 human fecal microbiomes using these genes as molecular markers, which revealed global population-specific distributions of Bacteroidaceae- and Prevotellaceae-mediated MLG utilization. Altogether, the data presented here provide new insight into the molecular basis of β -glucan metabolism in the HGM, as a basis for informing the development of approaches to improve the nutrition and health of humans and other animals.

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sciforum-123418: Thermal treatment of *Ribes magellanicum* reduces the polyphenol-mediated protection of the proteasome and increases the accumulation of glycated proteins in AGS cells exposed to methylglyoxal

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Accumulation of advanced glycation end products (AGEs) has been associated with cellular senescence. A decrease in proteasome activities have been observed in cells exposed to methylglyoxal. Polyphenols can scavenge methylglyoxal and also induce cellular protection against it through the activation of Nrf2-regulated enzymes. In this study, the efficiency of polyphenol-enriched extracts (PEEs) from the Chilean currant *Ribes magellanicum*, before and after thermal treatment (TT), was evaluated in terms of their ability to regulate proteasome expression and activity in AGS cells exposed to a challenge with methylglyoxal.

Polyphenols from *R. magellanicum* before and after TT (90°C, 1h) were extracted with different solvents: EtOH: acetic acid (99:1); EtOH: H₂O: acetic acid (75:24:1) and EtOH: H₂O: acetic acid (50:49:1). PEEs were characterized by means of HPLC-MS-MS and cyclic voltammetry. Gastric epithelial cells (AGS) were preincubated (16h) with PEEs before and after TT (from 31.25 to 250 µg/mL) and challenged with methylglyoxal (15 mM). Proteasome activities were determined by changes in the fluorescence of succinyl-Leu-Leu-Val-Tyr-amidomethylcoumarin (LLVY-AMC) and Z-Leu-Leu-Glu-AMC, for chymotrypsin-like activity (CTL) and peptidyl-glutamyl peptide hydrolyzing activity, respectively, in the presence (20 µM) and absence of the proteasome inhibitor N-Cbz-Leu-Leu-leucinal. Expression of proteasome subunits α-6, β-2, β-5, as well as the intracellular accumulation of argpyrimidine were determined by western blot analysis.

It was observed that PEEs extracted with EtOH: acetic acid (99:1) was the only extract able to protect CTL of the proteasome after the challenge with methylglyoxal. This fact was concomitant with the expression of the β-5 subunit of the proteasome. A decrease in the expression of the β-5 subunit of the proteasome was observed when cells were challenged with methylglyoxal. The accumulation of AGEs in proteins was higher in samples exposed to TT, in agreement with IC₅₀ of methylglyoxal scavenging induced by the PEEs. Our results indicate that TT of polyphenols induces oxidative modifications, decreasing their ability to protect the proteasome in AGS cells against a methylglyoxal challenge



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sciforum-122726: X-ray crystallography united with computational modelling reveal the catalytic mechanism in a hydrolytic enzyme

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Substrates and products bind and unbind in enzymes rapidly, which precludes investigations of their reactant trajectories. To address this gap, we solved over 40 crystal structures of plant exo-hydrolase, a glycoside hydrolase family 3 (GH3), and combined with kinetics, computational modelling and phylogenomics (1-3). Our data unveiled catalytic features and details of substrate binding and product unbinding at atomic levels. We showed that a non-covalently trapped glucose product evoked the formation of a transient lateral cavity, which served for its egress, and initiated the next catalytic cycle. This path enables substrate-product-assisted processivity, where the enzyme does not lose contact with oligo- or polymeric substrates (1). Evaluations of nanoscale reactant movements of the exo-hydrolase mutated in the Trp286/Trp434 clamp, proximal to the active site, defined reactant trajectories critical to binding and conformational behaviour of isomeric β -d-glucosides (2). We also defined the role of a non-nucleophilic E220 glutamate, essential for maintaining a water molecule network, but not involved directly in catalysis. We described a tightly harmonized network of concatenated water molecules near E220A and acid/base, and computational models disclosed the water molecules flux dynamics, which correlated with high catalytic efficiency in wild-type. This was contrary to the E220A mutant, in which this molecule network was disturbed (3). Ancestral sequence reconstruction of GH3 enzymes underscored the evolvability of a tryptophan clamp conferring substrate poly-specificity, and roles of residues underlining high catalytic efficiency, which correlated with characteristics of water-hydration sites (1-3). Our findings provide a blueprint for the mechanism and dynamics of catalysis facilitated by hydrolytic enzymes, which could inspire bioengineering to create a sustainable and interconnected bio-economy.

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Session 3. Membrane Proteins

sciforum-126081: Involvement of TRP channels in the butterfly wing eyespot determination.

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The color patterns of butterfly wings have long been a subject of interest among researchers. However, their fundamental pattern formation mechanisms remain unclear. It has been reported that a homolog of *TRPA1* gene, *painless*, is expressed at high levels in the prospective eyespot region during development, suggesting that TRPA1 is involved in the eyespot pattern determination. In this study, we conducted pharmacological experiments through the injection of chemical activators and inhibitors of TRP channels, which are multimodal receptors for temperatures, chemical ligands, and mechanical forces, during the critical period of the eyespot pattern determination. The injections were made into pupae immediately after pupation, and color pattern changes were quantified in emerging adult wings. We observed that some TRPA1 and TRPM7 inhibitors significantly enlarged the eyespot size.

In addition to the enlargement of the eyespot size, the ratio of the total eyespot area to the central pattern was also significantly increased with the same inhibitors.

These results suggest that TRPA1 and TRPM7 in the pupal wing tissues may be involved in the butterfly wing eyespot determination through changing calcium influx into epithelial cells, although natural activators of TRPA1 and TRPM7 in the pupal wing tissues are not clear at this point.



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sciforum-124646: Protein quality control mechanisms of CFTR as therapeutic targets for cystic fibrosis

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CFTR, a polytopic membrane protein, functions as a cAMP-regulated chloride channel expressed at the apical plasma membrane (PM) of epithelial cells. Mutations in CFTR cause cystic fibrosis (CF), one of the most common genetic diseases among Caucasians. The most prevalent CFTR mutation, $\Delta F508$ -CFTR, leads to protein misfolding, resulting in its ubiquitination and degradation by protein quality control mechanisms at both the endoplasmic reticulum (ER) and PM. Several ubiquitin (Ub) ligases involved in CFTR degradation have been identified. At the ER, CHIP, RNF5, RNF185, and HERC3 are associated with ER-associated degradation (ERAD) of misfolded CFTR. In contrast, CHIP, RFFL, and RNF34 are implicated in peripheral CFTR degradation at endosomes and the PM.

In recent years, combination therapies using CFTR modulators have shown promise in improving CFTR function and clinical outcomes. These modulators help correct CFTR misfolding and enhance its chloride channel activity at the PM. However, even in the presence of modulators, a portion of CFTR remains subject to ubiquitination and degradation, limiting therapeutic efficacy. Targeting CFTR-associated Ub ligases to selectively inhibit CFTR degradation presents a promising therapeutic strategy. In this talk, I will discuss recent advances in our understanding of CFTR protein quality control mechanisms and our current efforts to develop therapeutic approaches focused on modulating CFTR ubiquitination.



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sciforum-122908: Tetraspanin CD9 serves as a cellular receptor for fish Rhabdovirus eliciting clathrin- and caveolae-mediated endocytosis

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Fish rhabdoviruses are among the most widespread fish pathogens, causing fatal diseases in both wild and cultured fish worldwide. The envelope glycoproteins (G) of fish rhabdoviruses are responsible for binding to host cells, but the viral receptor(s) involved in this process have remained unknown. Here, we identified *Lateolabrax japonicus* CD9 (LjCD9), a member of the tetraspanin family, as an entry receptor for viral hemorrhagic septicemia virus (VHSV), the type species within the Rhabdoviridae family. Overexpression of LjCD9 facilitates VHSV entry, whereas knock-down of LjCD9 had the opposite effects. We also demonstrated a direct interaction between LjCD9 and VHSV G, with the G protein domain IV and the LjCD9 EC2 domain being responsible for this interaction. Blocking LjCD9 with antibodies or purified proteins notably reduced VHSV entry into cultured cells. LjCD9 protein and anti-CD9 antibodies effectively enhanced the survival curve of VHSV-infected *L. japonicus*. Exogenous expression of LjCD9 independently facilitated the internalization of VHSV into VHSV-negative cells. Additionally, we successfully identified nitazoxanide as a potent inhibitor of VHSV entry by disrupting the VHSV G-LjCD9 interaction. Furthermore, LjCD9 interacted with another fish rhabdovirus, SCR (Siniperca chuatsi Rhabdovirus), G protein and facilitated SCR entry into HEK293T cells, indicating that LjCD9 acts as a cross-genus functional receptor for fish Rhabdoviruses. Finally, we found that CD9 mediated VHSV entry via clathrin- and caveolae-mediated endocytosis. In summary, our findings identified a novel receptor responsible for facilitating the entry of Rhabdoviruses into host cells via clathrin- and caveolae-mediated endocytosis in fish and revealed CD9 as a potential therapeutic target for Rhabdoviruses in fish.



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sciforum-123115: Voltage dependence of G protein coupled receptors

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G-protein coupled receptors (GPCRs) mediate the vast majority of signal transduction processes in the body. Although GPCRs span the cell membrane, they traditionally were not considered to be voltage dependent. In recent years, it was found that the affinity and activity of several GPCRs are regulated by membrane potential. The most studied voltage sensitive GPCRs are the muscarinic receptors. We found that the affinity of these receptors toward acetylcholine is voltage sensitive; the affinity is lower at resting potential than under depolarization for the M1R and higher at resting potential than under depolarization for the M2R. We further showed that this voltage sensitivity is an intrinsic property of the receptor as it was shown that depolarization induces charge movements in these receptors, analogous to gating currents found in voltage gated ion channels. We further identified a novel voltage sensing motif in the M2R that may be responsible for the voltage dependence of this receptor. More recent studies from our laboratory, as well as from others, demonstrated that voltage-dependence may be a general property common to many GPCRs. I will discuss our recent finding regarding the molecular mechanism that underlies the voltage dependence, as well as the physiological role of this property of GPCRs.



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sciforum-123271: Dysregulation of Multiple Solute Carrier genes and Metabolic Deficits in SLC1A4-Mutant Human iPSC-Derived Hippocampal Neurons

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Mutations in *SLC1A4*, which encodes the neuronal amino acid transporter ASCT1, impair metabolic and synaptic homeostasis, contributing to neurodevelopmental deficits and autism. To elucidate the molecular mechanisms underlying *SLC1A4*-related disorders, we employed an integrated multi-omics approach using human iPSC-derived hippocampal neurons. This approach combined electrophysiology, calcium imaging, metabolomics, proteomics, and transcriptomics. Our results reveal a biphasic neuronal phenotype: an early phase characterized by neuronal hyperexcitability, driven by increased sodium and potassium currents, followed by a progressive decline in synaptic activity at later stages. Metabolomic profiling during early differentiation revealed elevated levels of glycine, serine, and glutamate, indicative of excitotoxic stress. In contrast, the later phase was marked by glutamate depletion and disruption of the extracellular matrix (ECM), correlating with impaired synaptic function. Proteomic analyses showed early-stage dysregulation of metabolic pathways, including amino acid biosynthesis and fatty acid metabolism, while later stages were marked by abnormalities in metabolic and ECM-receptor interaction pathways. Transcriptomic profiling further revealed alterations in calcium signaling and amino acid metabolism pathways, including valine, leucine, and isoleucine degradation, tryptophan metabolism, and glycine-serine-threonine metabolism. Additionally, analysis of SLC-family transporter genes revealed disruptions in glutamate and glycine transport, directly linking amino acid transport deficits to neuronal dysfunction. Taken together, our findings demonstrate that *SLC1A4* mutations disrupt multiple solute carrier genes, leading to metabolic stress, altered neuronal excitability, and synaptic abnormalities. This work provides a molecular framework for understanding *SLC1A4*-related neurodevelopmental disorders and highlights potential therapeutic targets.



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sciforum-122904: Effect of γ -secretase dysfunction on neuronal cholesterol metabolism

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Introduction

Dysfunction of a membrane protease, γ -secretase, which has presenilin 1 (PS1) or PS2 as catalytic subunit, has a significant impact on Alzheimer disease (AD) pathogenesis. We have recently reported that PS1- or PS2-knock out (KO) results in intracellular cholesterol accumulation via impairment of protein glycosylation and function of NPC1 playing a role in intracellular cholesterol transport (Fabiano et al., *Int. J. Mol. Sci.*, 2024). However, it remains elusive how γ -secretase dysfunction can affect neuronal cholesterol metabolism.

Methods

To examine the effect of γ -secretase dysfunction on NPC1 expression, several non-neuronal cells, such as mouse embryonic fibroblasts (MEFs) and human fibroblasts, and neuronal cells, like differentiated SH-SY5Y cells and human iPSC cell-derived neurons (iPSC-Ns), were treated with γ -secretase inhibitors. Then, cell membrane fractions were collected and analyzed by Western immunoblotting.

Results

As in our recent report, treatment with a γ -secretase inhibitor, DAPT, did not decrease NPC1 expression not only in MEFs but also in other non-neuronal cells. On the other hand, treatment with γ -secretase inhibitors reduced NPC1 expression in the neuronal cells examined.

Conclusions

Dysfunction of γ -secretase could have different effects on intracellular cholesterol transport in non-neuronal and neuronal cells. Since NPC1 reduction causes neurodegeneration in Niemann-Pick disease type C, it is necessary to further evaluate and clarify molecular mechanism underlying the NPC1 reduction by γ -secretase dysfunction in neurons.



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sciforum-124329: Effects of Voltage Dependence and Rate Constants on Transient Currents in a Thermodynamically Constrained Mathematical Model of the Na/Ca Exchanger

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Specialized mathematical models have been proposed to quantitatively assess the ion transporter performance in heart-related physiological experiments. The Na/Ca exchanger imports three Na⁺ ions into the cell and exports one Ca²⁺ ion; therefore, it plays a vital role in cardiomyocyte ion homeostasis. The detailed characteristics of the voltage and ion concentration dependencies of the exchanger current were reported by Matsuoka and Hilgemann in 1992, whereas existing mathematical models can only reproduce a limited range of experimental data. This study primarily focuses on the development of a new mathematical model by introducing charge movements to all consecutive state transition processes under thermodynamic constraints, which accomplish voltage dependencies and current generation for each ion binding and dissociation process. The proposed model includes 22 charge movement, dissociation constant, and rate constant parameters, which were optimized to fit the voltage and ion concentration dependencies of the steady-state transporter currents. Through this process, most parameters converged effectively. Using the optimal parameters, our model successfully replicated the experimental steady-state current data as well as the transient current data. In addition to validating the voltage-dependent behaviors, we further investigated how ion-binding reaction rates and ion concentration rise times affect transient current amplitudes. Our results show that faster reaction rates and slower concentration changes attenuate the peak transient currents, providing deeper kinetic insights into transporter function.



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sciforum-123167: Functional analysis of HECT-type E3 ligase HERC3 in ER-associated degradation of CFTR mutants.

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CFTR (Cystic Fibrosis Transmembrane conductance Regulator) is a chloride ion channel that causes cystic fibrosis (CF). The $\Delta F508$ CFTR variant, carried by approximately 90% of patients with CF, is misfolded and degraded by ERAD, resulting in reduced expression on the plasma membrane and causing CF pathology. Previous studies have identified RNF5 and RNF185 (RNF5/185) as ERAD-related E3 ubiquitin ligases for CFTR mutants. In this study, we investigated the function of HERC3, a cytoplasmic HECT-type E3 ligase that we newly identified as a CFTR-related E3 ligase. We have established new evaluation systems using HiBiT luminescent tags and LgBiT to quantify the kinetics of ERAD-mediated CFTR degradation and retro-translocation in real-time. RNF5/185 double KO cell-based HiBiT ERAD assay revealed that HERC3 facilitates ERAD of CFTR mutants even in the absence of RNF5/185. Further investigation of CFTR domain mutants showed that HERC3 promotes ERAD of both CFTR transmembrane domains (MSD1 and MSD2) individually. In addition, in vitro binding experiments using a cell-free protein synthesis system revealed that HERC3 binds to CFTR MSDs exposed from the lipid membrane, but not to CFTR MSDs embedded in the lipid membrane. Consequently, we propose that HERC3 is involved in ER quality control by selectively recognizing the transmembrane region of CFTR exposed from the ER membrane and facilitating ERAD.



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sciforum-113240: Indoxyl sulfate, a uremic toxin, inhibits myoblast differentiation by the imbalance of cellular iron metabolism and reactive oxygen species

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Introduction

The accumulation of uremic toxins is common among chronic kidney disease (CKD) patients. Indoxyl sulfate (IS), one of the primary uremic toxins, has been indicated to be associated with sarcopenia. Iron plays a crucial role in muscle growth and differentiation, with ferrous ions contributing to intracellular oxidative stress. Both iron excess and deficiency can affect muscle growth. While IS can affect muscle regeneration and differentiation, the mechanisms involved are poorly understood. We hypothesized that IS affected myoblast differentiation through its impact on the balance of intracellular oxidative stress and iron metabolism.

Methods

Experimental design involved exposing mouse C2C12 myoblasts and primary human skeletal myoblasts to IS during the proliferation phase and continuing exposure throughout the differentiation process.

Results

The results showed that IS reduced intracellular reactive oxygen species (ROS) and free Fe²⁺ levels during differentiation. IS promoted the changes in intracellular iron metabolism including that enhanced the expression levels of the ferrous ion import transporter (DMT1) and the export transporter (ferroportin), but did not affect the protein expression of transferrin, a ferric iron (Fe³⁺)-binding protein, and CD71, a transferrin receptor. IS could also increase the gene expression levels and activities of antioxidant-related enzymes, maintaining cells in a high-antioxidant state to achieve a new balance. Unexpectedly, supplementation with Fe²⁺ (FeSO₄) with or without IS significantly increased ROS levels, but exacerbated the inhibition of myoblast differentiation induced by IS, indicating the cellular redox balance was disrupted.

Conclusions

These findings reveal that IS induces an imbalance of cellular iron metabolism and oxidative stress, providing new insights into an alternative mechanism by which IS inhibits muscle regeneration and differentiation.



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sciforum-126212: Integrins may regulate epithelial cell adhesion to cuticle for color pattern development in butterflies

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The insect cuticle is an extracellular matrix (ECM) primarily composed of the polysaccharide chitin and cuticular proteins. In butterflies, the wing color pattern is formed by the arrays of various types of pigmented and structural scales. During the early pupal stage, a cuticle layer adheres to the epithelial cells that will later differentiate into scale cells. In the blue pansy butterfly (*Junonia orithya*), the prospective eyespot pattern is pre-determined early in pupation, and the center of the future eyespot—recognized as a developmental organizer—exhibits a thicker cuticle layer, compared to surrounding regions. Based on these observations, we hypothesize that the cuticle may function as a regulatory interface influencing epithelial cell fate decisions for terminal differentiation. Previous studies have demonstrated that modulating cuticle stiffness alters wing color patterns: softer and harder cuticles lead to smaller and larger eyespots, respectively. Given the importance of the cellular adhesion to cuticle in color pattern determination, integrins may play a key role as adhesion receptors. In this study, we pharmacologically treated the pupal wing tissues with integrin activators and inhibitors and examined whether adult wing color patterns were modified. We also conducted real-time in vivo imaging of the pupal wing tissues to assess how variations in adhesion strength may affect the wing color patterns in butterflies.



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sciforum-117021: MT1-MMP: new therapeutic target of metabolic disorder

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Obesity is a global health epidemic that increases the risk of developing life-threatening conditions such as diabetes and heart disease. The cytokine GDF15 and its cognate receptor GFRAL, expressed in neurons in the area postrema and solitary tract nucleus, are responsible for regulating appetite and body weight. Individuals with obesity have increased plasma levels of GDF15; however, they are resistant to its anorexigenic effects. Our recent study has uncovered a role for the matrix metalloproteinase MT1-MMP (also known as MMP14) in the negative regulation of GDF15–GFRAL signaling in the context of obesity, revealing a new regulatory mechanism that underpins the sensation of fullness, or satiety (**Nature Metabolism, 2022**). In addition, we have identified a new proteolytic event mediated by MT1-MMP in the control of insulin sensitivity (**Nature Communications, 2022**), revealing a new mechanism underlying insulin resistance in the context of ageing. Through in silico drug screening, we have identified a key compound from herbal medicine for the management of obesity by targeting the GDF15–GFRAL signaling axis (**Nature Communications, 2024**). Our findings identify a regulatory mechanism for the control of non-homeostatic body weight regulation, provide mechanistic insights into the regulation of insulin sensitivity, and highlight MT1-MMP as a promising target for multiple metabolic disorders.



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sciforum-123135: Novel Insights into Intracellular P-Type ATPase Functions in Disease

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P-type ATPases, including sodium pumps (Na^+, K^+ -ATPases), the gastric proton pump (H^+, K^+ -ATPase), and Ca^{2+} pumps (Ca^{2+} -ATPases), play a crucial role in maintaining electrochemical gradients and cellular homeostasis. Dysregulation of their expression and function has been associated with various diseases, including cancer and neurodegenerative disorders. Here, we explore the physiological and pathological roles of two intracellular P-type ATPases: the Na^+, K^+ -ATPase $\alpha 3$ -isoform ($\alpha 3\text{NaK}$) in cancer cells and ATP13A2 (also known as PARK9) in neurons. $\alpha 3\text{NaK}$ is normally localized in the plasma membrane of neurons, skeletal muscle, and cardiac muscle. However, we observed aberrant intracellular vesicular expression of $\alpha 3\text{NaK}$ in various human cancer cell types. Upon cancer cell detachment, $\alpha 3\text{NaK}$ translocates to the plasma membrane, a dynamic shift that appears to facilitate the survival of detached (metastatic) cancer cells. Inhibition of intracellular $\alpha 3\text{NaK}$ by cardiac glycosides, potent Na^+, K^+ -ATPase inhibitors, is found to suppress cancer cell growth and metastasis. Interestingly, $\alpha 3\text{NaK}$ has been found to localize in the plasma membrane of human circulating tumor cells, which contribute to metastasis by traveling through the bloodstream. Conversely, ATP13A2 is implicated in Kufor-Rakeb syndrome, an autosomal recessive early-onset form of Parkinson's disease. Our recent findings reveal that ATP13A2 functions as a novel H^+, K^+ -ATPase within neuronal lysosomes, exhibiting distinct pharmacological properties. Its H^+/K^+ transport activity is inhibited by potassium-competitive acid blockers (P-CABs; SCH28080 and vonoprazan) and the SERCA Ca^{2+} -ATPase inhibitor thapsigargin. Mutations identified in Parkinson's disease patients result in decreased ATP13A2 expression and activity. Inhibition of ATP13A2 leads to lysosomal alkalinization and α -synuclein accumulation in neuronal cells. These findings highlight novel roles of intracellular P-type ATPases in refractory diseases, offering potential avenues for therapeutic intervention.



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sciforum-126250: Novel roles of epithelial ion channels in endocrine functions

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Cystic fibrosis transmembrane conductance regulator (CFTR) and the epithelial Na⁺ channel (ENaC) are two typical ion channels abundantly expressed in epithelial tissues. Their primary functions are believed to mediate electrolyte-transport across the epithelium, which drives water movement to realize epithelial fluid secretion/absorption in epithelial cell-enriched organ-systems such as the airway, gastrointestinal and reproductive tracts. Mutation or malfunction of CFTR or ENaC results in diseases such as cystic fibrosis. Interestingly, our previous work demonstrated these two ion channels to have versatile roles beyond epithelial fluid transport and recent evidence showed CFTR and ENaC to be expressed in non-epithelial tissues as well such as endocrine cells. For the recent years, we have adopted primarily Cre-LoxP-based conditional knockout transgenic mouse models and provided experimental data to suggest direct involvement of CFTR and ENaC in the functions of endocrine cells such as ovarian granulosa cells, osteocytes and pancreatic islet cells, which are critical to several physiological processes including ovarian steroidogenesis, bone remodeling and glucose metabolism. We also investigated the underlying molecular mechanisms using experimental approaches including electrophysiology, live-cell Na⁺/Cl⁻/Ca²⁺/cAMP imaging, in vivo fluorescence imaging, as well as transcriptional and proteomic analysis. Collectively, these findings have provided new insights into the understanding of endocrine systems as well as related human disorders. *(The work was supported in part by Areas of Excellence (No. AoE/M-402/20), General Research Fund (No.15102622, No.15106423) from The Research Grant Council of Hong Kong, and Health and Medical Research Fund of Hong Kong (No. 18191361)).*



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sciforum-125567: Oxymatrine alleviates severe acute pancreatitis by inhibiting the excessive formation of NETs via the LFA-1/PKC/ROS pathway in neutrophils

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Severe acute pancreatitis (SAP) is a highly fatal inflammatory disease of the digestive system with limited treatment options as a result of the complex etiology. Although the molecular mechanism of SAP is still not fully understood, excessive formation of neutrophil extracellular trap (NETs) has a key role in pancreatic and multiple organ injuries. Herein, we found that oxymatrine (OMT), main ingredient extracted from the roots of *Sophora flavescens Aitona* initially prescribed for Chronic hepatitis B, alleviated the pathological changes of pancreas induced by sodium taurocholate in mice, inhibited local and systemic inflammation and alleviated multiple organ dysfunction. Network pharmacology and proteomic analysis showed that the formation of NETs is the key signal pathway of OMT in the treatment of SAP, and the decrease of NETs structure in mouse pancreas after OMT intervention supports this conclusion. Subsequently, *in vitro* studies further confirmed that OMT inhibited NE nuclear translocation and increased the nuclear HDAC level by regulating the LFA-1/PKC/ROS signaling pathway of neutrophils to inhibit the excessive formation of NETs and alleviate the severity of SAP. These findings suggest that OMT might attenuate pancreatic and distant organ damage in SAP, potentially through inhibition of NETs formation via the LFA-1/PKC/ROS pathway, underscoring its potential therapeutic application for SAP management.



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sciforum-122423: Sirtuin-2 plays a key role in improving age-related decline in skin barrier function

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Introduction

Claudin-1 (CLDN1) and CLDN4 are highly expressed in keratinocytes and may function as paracellular barrier to water and small molecules. Although numerous reports have been implicated CLDN1 in allergies and inflammatory diseases, the role of CLDN4 has not yet been fully elucidated. Therefore, we investigated the relationship between aging and CLDN4.

Methods

Skin tissues extracted from C57BL mice and human epidermis-derived keratinocyte HaCaT cells were used in the experiment. mRNA and protein expression levels were measured by real-time PCR and Western blotting, respectively. Cellular localization of protein was confirmed by a fluorescence immunostaining. siRNA was used for knockdown of target molecules. Intercellular barrier function was evaluated by transepithelial electrical resistance (TER) and permeability to the low molecular weight fluorescent dye lucifer yellow (LY). Sirtuin-2 (SIRT2) activity was measured using an ELISA kit.

Results

The protein and mRNA levels of CLDN4 were significantly decreased in the skin tissues of old mice (36-weeks old) compared with young mice (6-weeks old). SIRT2 activity was significantly reduced in old mice compared with young mice. The levels of CLDN4 protein and mRNA were significantly reduced by tenovin-1 (Ten-1), a SIRT2 inhibitor, in HaCaT cells. Skin barrier function was also decreased by Ten-1 treatment. These results were consistent with the results of SIRT2- and CLDN4-silencing cells. Since the relationship between SIRT2 and CLDN4 was suggested, we searched for activators of SIRT2. We found several SIRT2 activators using a library of approximately 220 food compounds. Among them, curcumin rescued the decline in SIRT2 activity and paracellular barrier function caused by Ten-1 in HaCaT cells.

Conclusion

We revealed that aging may reduce skin barrier function mediated through the decrease in SIRT2 activity, increase in CLDN4 acetylation, and decrease in CLDN4 expression. Furthermore, curcumin can improve barrier function by increasing SIRT2 activity. These data provide us with a clue for the development of SIRT2-targeting drugs for the prevention of xerosis senile.



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sciforum-123154: Targeting TRPM7 with Carvacrol to Alleviate Neuroinflammation in Alzheimer's Disease: Mechanistic and Therapeutic Insights

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Introduction

Maintaining physiological calcium (Ca²⁺) homeostasis is crucial in modulating neuroinflammation in Alzheimer's disease (AD). Recent studies highlight the transient receptor potential melastatin-7 (TRPM7) channel as a key regulator of inflammatory responses, particularly in macrophages, where its activation during inflammation acts as a molecular switch. Given TRPM7 is abundantly expressed in the central nervous system, this channel may similarly influence neuroinflammatory processes. Here, we investigate the therapeutic potential of TRPM7 inhibition using carvacrol, a natural compound derived from traditional Chinese medicine, to suppress neuroinflammation in AD models.

Methods

We assessed neuroinflammatory responses in mouse and human microglia using lipopolysaccharides (LPS) or interferon-gamma (IFN- γ) to induce inflammation. TRPM7 inhibition was achieved with carvacrol and microglia-specific AAV-shTRPM7. Flow cytometry and phenotypic analysis were used to evaluate microglial polarization. *In vivo* efficacy was tested in the 5xFAD AD mice treated with carvacrol or AAV-shTRPM7.

Results

LPS treatment elevated cytoplasmic Ca²⁺ levels and promoted proinflammatory microglial phenotypes. TRPM7 inhibition with carvacrol reduced Ca²⁺ levels and attenuated LPS-induced neuroinflammation. Flow cytometry analysis revealed that carvacrol shifted microglia from a pro-inflammatory M1-like state to an anti-inflammatory M2-like state. *In vivo*, TRPM7 inhibition enhanced amyloid plaque clearance and improved cognitive function in 5xFAD AD mice.

Conclusion

TRPM7 plays a pivotal role in regulating intracellular Ca²⁺ homeostasis and microglial polarization in AD. Our findings demonstrate that TRPM7 inhibition with carvacrol mitigates neuroinflammation and promotes amyloid clearance, highlighting TRPM7 as a promising therapeutic target for neuroinflammatory conditions associated with neurodegeneration. Further development of TRPM7 inhibitors offers novel strategies for AD treatment.

Acknowledgement

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Session 4. Cancer Target Proteins

sciforum-138346: Novel functions of aquaporins in cancer and their potential as therapeutic targets

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Aquaporins (AQPs) are transmembrane proteins that facilitate the transport of water and small molecules across the plasma membrane, playing key roles in water homeostasis. Beyond their physiological functions, AQPs are aberrantly expressed in tumor cells and tissues of diverse origins, particularly in aggressive cancers. Recent studies have indicated that AQPs are involved in cell migration, proliferation, and apoptosis, suggesting that they may serve as novel therapeutic targets in cancer treatment.

Among the 13 known isoforms, AQP1 is widely expressed in tumor vascular endothelium and is involved in tumor angiogenesis by promoting endothelial cell migration. This AQP1-dependent migration is thought to be due to water influx into the lamellipodia at the leading edge of migrating cells.

AQP5, on the other hand, is found in breast and pancreatic cancers, as well as in lung adenocarcinoma, and has been shown to influence not only cell migration but also proliferation and apoptosis. AQP5 expression has been reported to enhance the steady-state phosphorylation levels of MEK/ERK, which may contribute to increased cell proliferation. Furthermore, AQP5 has been shown to reduce reactive oxygen species (ROS) accumulation and suppress apoptosis in response to various toxic stimuli. Interestingly, the AQP5-mediated increase in proliferation and decrease in apoptosis appear to be independent of its water-transporting function.

Therefore, targeting these novel, non-canonical functions of AQPs in tumor tissues may represent a promising strategy for cancer therapy. In this presentation, I will highlight the anti-apoptotic and antioxidant effects of AQP5, as well as the inhibitory effects of traditional herbal medicines on AQP function.



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sciforum-124429: The role of ferroptosis in gastrointestinal cancers and development of novel therapeutics targeting ferroptosis.

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Ferroptosis, a recently recognized form of programmed cell death, has emerged as a critical pathological mechanism across multiple medical fields, including ischemia reperfusion injury, neurodegenerative disease, and cancer. Characterized by iron dependent reactive oxygen species (ROS) production and lipid peroxidation, ferroptosis represents a novel and promising therapeutic target. In my previous work, we investigated the role of ferroptosis in liver ischemia reperfusion injury using a novel normothermic machine perfusion platform named PROTECT (Perfusion Regulated Organ Therapeutics with Enhanced Controlled Testing) developed at Saint Louis University. This unique platform employs human discarded split liver and dual ECMO circuits to recapitulate liver ischemia reperfusion injury under controlled ex vivo conditions, enabling rigorous internal comparisons and therapeutic testing. Using this platform our team demonstrated that regulation of iron metabolism with an iron chelator attenuates iron accumulation, lipid peroxidation, and ferroptosis-related gene expression, highlighting its potential as a robust therapeutic strategy for liver ischemia reperfusion injury.

Given the pivotal role of oxidative stress in ferroptosis, we further explored its role in cancer biology. Tumor cells face severe hypoxic and oxidative stress due to uncontrolled cell proliferation and enormous energy consumption. For tumor promotion, tumor cells are required to survive under that severe condition and resulting in driving some antioxidant pathways. Notably, some anti-cancer drugs such as cisplatin or rapamycin, can induce ferroptosis, whereas chemotherapy-resistant tumors develop mechanisms to evade ferroptotic cell death. Targeting ferroptosis, therefore, offers a promising strategy to disrupt tumor survival under oxidative conditions and restore chemotherapy sensitivity. Our previous studies investigating ion channels and transporters in various cancers have revealed the critical involvement of ion metabolism in cancer progression and therapeutic resistance. Building upon this foundation, we aim to develop innovative therapeutic approaches that modulate ferroptosis by targeting the transport of ions and amino acids in cancer cells, thereby providing a new framework for overcoming therapy resistance and improving clinical outcomes.



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sciforum-123055: 20(S)-protopanaxadiol inhibits pancreatic cancer progression through targeting the C-X-C chemokine receptor type 4

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with limited therapeutic options and poor clinical outcomes. This study investigates the anticancer potential of 20(S)-protopanaxadiol (PPD), a bioactive ginseng derivative, focusing on its direct targeting on the CXCR4. In vitro experiments demonstrated that PPD significantly inhibited PDAC cell proliferation by inducing G1-phase cell cycle arrest and reducing clonogenic capacity. Morphological and molecular analyses revealed that PPD primarily triggered pyroptosis through activation of cleaved gasdermin D (GSDMD) and gasdermin E (GSDME), rather than apoptosis. Transcriptomic analysis and molecular docking identified CXCR4 as a direct target of PPD, with downregulation of CXCR4 expression disrupting tumor-promoting pathways. Additionally, PPD remodeled the tumor micro-environment (TME) by modulating cancer-associated fibroblasts (CAFs) activity and suppressing CXCL12 secretion in CAFs. Furthermore, in vivo studies using mouse xenograft models demonstrated significant reductions in tumor volume and weight with minimal systemic toxicity.

The findings establish PPD as a promising therapeutic candidate for PDAC through dual mechanisms: firstly, by reducing CXCR4 expression to induce PDAC cell pyroptosis; secondly, by inhibiting CXCR4-CXCL12 signaling axis between CAFs and cancer cell, thereby reconstructing the TME to enhance PPD penetration into PDAC tissue. The multi-level regulation of the CXCR4 by PPD provides a novel therapeutic strategy for PDAC treatment.



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sciforum-123262: Antitumor effect and mechanism of 3-Methylindole and its derivative epoxydibenzonaphthyridine (O-DBNT) in MDA-MB-231 breast cancer cell line

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Background

Breast cancer ranks first in incidence and fifth in cancer-related mortality worldwide. Among its subtypes, triple-negative breast cancer (TNBC) remains particularly challenging due to treat its high recurrence rate following surgery and resistance to conventional therapies, highlighting the urgent need for novel treatment strategies. Indole alkaloids, a class of natural nitrogen-containing compounds, are known for their potent antitumor and anti-inflammatory properties. Recently, indole derivatives have garnered increasing interest for their potential in cancer therapy. This study will investigate the antitumor effects and underlying mechanisms of 3-methylindole and its derivative O-DBNT on MDA-MB-231 triple-negative breast cancer cells.

Methods

MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with fetal bovine serum and penicillin/streptomycin. Cells were treated with four concentrations (0.5, 0.75, 1.5, and 3 mM) of 3-methylindole or O-DBNT. Cell viability was assessed, and flow cytometry was used to evaluate apoptosis, cell cycle distribution, and oxidative stress.

Results

Both compounds significantly reduced MDA-MB-231 cell viability. O-DBNT demonstrated a stronger inhibitory effect at lower concentrations and shorter exposure times, while 3-methylindole exhibited greater inhibition at higher concentrations and longer exposure. Cell cycle analysis revealed that O-DBNT increased the Sub-G1 population and reduced the S-phase population in a dose-dependent manner, whereas 3-methylindole induced cell cycle arrest in the G2/M phase. Apoptosis assays confirmed that both compounds induced apoptosis in a concentration-dependent manner.

Conclusion

3-Methylindole and O-DBNT effectively inhibit the growth of MDA-MB-231 TNBC cells by promoting apoptosis, inducing cell cycle arrest, and increasing oxidative stress. These findings support the potential of both compounds as candidates for future development in cancer therapeutics.



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sciforum-126448: Application of Kampo medicines for the palliation of cancer cachexia

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Cancer cachexia results in the discontinuation of aggressive cancer therapy, and halting its progression has a significant effect on the survival rate and quality of life of patients with cancer. Currently, there are few therapies to control or slow down the progression of cancer cachexia. Although traditional Japanese Kampo medicine is widely used to support aggressive cancer therapy, the relevant scientific evidence is limited. Additionally, Kampo medicines are based on historical experience. In recent years, there have been widespread attempts to prove the efficacy of Kampo medicines through basic research, and an increasing number of studies have clarified the mechanism of action of Kampo medicines at the molecular level. It has been proposed that the improvement of cancer cachexia by Kampo medicines might involve enhancement of feeding via the central nervous system, improvement of protein maintenance in the skeletal muscle, and suppression of inflammatory cytokine production. In particular, among Kampo medicines, tonifying formulae, called “hozai” in Japanese, have been shown to be effective in alleviating cancer cachexia. In this review, we summarize the recent progress of basic and clinical research in Kampo medicines on cancer cachexia, and introduce Kampo medicines that are expected to be an attractive supportive cancer medication.



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sciforum-126183: Casein Kinase II (CK2) regulates cell cycle progression and chromatin remodeling in hematological malignancies

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Casein kinase II (CK2) is a pro-oncogenic kinase, which is overexpressed in most human malignancies. CK2 promotes cellular proliferation and malignant transformation via phosphorylation of a large number of substrates. We have previously demonstrated that CK2 directly phosphorylates tumor suppressor protein - IKAROS and that IKAROS phosphorylation by CK2 impairs its function as transcriptional regulator and tumor suppressor. These findings identified CK2-IKAROS signaling axis, which controls proliferation of malignant cells and drug resistance in leukemia. Here, we describe the role of CK2-IKAROS axis in regulation of cell cycle progression in different types of human hematological malignancies. Analysis of global genomic occupancy of Ikaros in primary cells of acute lymphoblastic leukemia, (ALL), acute myeloid leukemia (AML) and B-cell lymphoma showed a strong DNA binding of Ikaros to the promoters of a large number of genes that control G1, G2, and M cell cycle checkpoints. This was confirmed by qChIP in primary cells. The role of IKAROS in transcriptional regulation of the cell cycle-promoting genes was tested using gain-of-function and loss-of-function experiments. Results showed that IKAROS is a transcriptional repressor of the genes that control G1/S and G2/M transition. We tested the effect of CK2 on IKAROS' transcriptional regulation of the cell cycle-promoting genes in human hematological malignancies. Molecular and pharmacological inhibition of CK2 with a specific inhibitor, CX-4945, resulted in increased IKAROS binding to the promoters, alterations in chromatin remodeling, and transcriptional repression of the cell cycle-promoting genes. This was associated with the cell cycle arrest of human ALL, AML and lymphoma cells. Pharmacological treatment with CK2 inhibitor exerted a strong therapeutic effect in preclinical models of human ALL. In conclusion, these results demonstrate that CK2-IKAROS signaling axis has a critical role in transcriptional regulation of the cell cycle-promoting genes and cell cycle progression. Targeting CK2 is a promising treatment for human hematological malignancies.



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sciforum-121516: Design of potent elongated *m*-Terphenylpicolinamide derivatives and their interactions with the PD-L1 protein

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Introduction

Programmed Cell Death Protein-1 (PD-1)/Programmed Cell Death-Ligand 1 (PD-L1) interaction has a crucial role in maintaining the immune system's self-tolerance by downregulating T cell activation. This mechanism is also used by several types of cancers. By overexpressing the PD-L1 protein, they can evade the immune response and, therefore, become invisible to the immune system. Herein we present a novel class of small molecular inhibitors targeting PD-L1 protein.

Methods

In our studies, we focused on in vitro-based SAR analysis of the found scaffold using Jurkat-ECs co-cultured with CHO/TCRAct/hPD-L1 cells. We docked the devised terphenyl ligands onto PD-L1 protein taken from PDB 6VQN crystal structure. After chemical synthesis, the most promising inhibitor was then tested on the co-culture of PBMC with CHO/TCRAct/hPD-L1. Diffraction-quality crystals of the PD-L1/ligand complex were obtained by using a sitting-drop setup. The final resolution of the obtained cocrystal structure was 1.75 Å. The protein interactions with the ligand were analyzed in PyMol.

Results

The best synthesized SMIs achieved EC₅₀ in the 14–40 nM range, on par with best known inhibitors of PD-L1, and presenting a striking increase from previous short structures. Elongation of the scaffold provided beneficial interactions with the protein and highlighted the role of the picolinamide moiety. The bioassay tests indicated a significant increase in the activation of the T cell population and cytotoxic properties toward cancer cells. Furthermore, treated blood lymphocytes could efficiently eliminate cancer cells from the co-culture.

Conclusions

The PD-L1 protein displays an unique and flat hydrophobic binding pocket which provides a challenge for design of small-molecule inhibitors. In our study, we were able to rationalize the binding of the small molecule to the PD-L1 protein. Developed N-terphenylpicolinamides display high affinity to the molecular target. The described compounds are promising drug candidates.



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sciforum-124101: Deubiquitinating enzyme-mediated regulation of the pro-apoptotic protein Bax

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Introduction

Bax, a pro-apoptotic member of the Bcl-2 family, plays a pivotal role in mitochondrial apoptosis by permeabilizing the outer mitochondrial membrane. Although its function has been extensively studied, the mechanisms underlying its post-translational regulation remain poorly understood. Recent studies suggest that deubiquitinating enzymes (DUBs) may fine-tune apoptotic signaling by modulating the ubiquitination status of key regulatory proteins. Here, we investigated whether DUBs contribute to Bax regulation under different cellular stress conditions.

Methods

We conducted yeast two-hybrid screening using the full-length Bax to identify DUB interactors, followed by validation through immunoprecipitation and GST pull-down assays. The effect of DUBs on Bax ubiquitination was examined under conditions of DUB overexpression or pharmacological inhibition. Apoptotic responses were evaluated using flow cytometry after staurosporine (STS), hydrogen peroxide (H₂O₂), or UV treatment.

Results

Through this approach, we identified two DUBs (DUB-Bax1 and USP12) that bind directly to Bax and modulate its ubiquitination. Notably, the inhibition of DUB-Bax1 led to increased ubiquitination levels of Bax, indicating its active role as a DUB. However, overexpression of these enzymes did not significantly impact Bax protein stability, suggesting a modulatory role rather than a protective one. Interestingly, under oxidative and genotoxic stress conditions (H₂O₂ or UV), but not under STS-induced apoptosis, overexpression of DUB-Bax1 markedly enhanced apoptotic cell death. In the meantime, USP12 is involved in a proteasome-independent process, suggesting that it may regulate other cellular functions rather than protein stability.

Conclusion

Taken together, our findings demonstrate that DUB-Bax1 and USP12 regulate Bax activity through deubiquitination in a stimulus-dependent manner. These enzymes may act as key modulators of apoptosis in response to oxidative or DNA-damaging stress and could represent promising therapeutic targets in diseases characterized by defective apoptotic signaling. Future studies should focus on mapping the specific ubiquitination sites targeted by DUB-Bax1 and evaluating its functional relevance in *in vivo* models.



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sciforum-123040: EPA and DHA from *Decapterus macarellus* Suppresses Colorectal Cancer Cell Proliferation and Apoptosis via TP53 and NF- κ B Pathway Modulation

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The increasing number of colorectal cancer (CRC) patients and mortality rates demand alternative therapies with fewer side effects than conventional chemotherapy and surgery. Mackerel scad (*Decapterus macarellus*) offers promising sources of fatty acids especially EPA and DHA with potential anticancer properties. This study investigates the cytotoxic and pro-apoptotic effects of skin oil on WiDr colon cancer cells and explores its molecular mechanism through integrative in vitro and silico approaches. Cytotoxicity tests were performed using the MTT assay, revealing selective inhibition of WiDr cells (IC₅₀ = 958.04 μ g/mL) and low toxicity against normal Vero cells. Flow cytometry analysis showed that the oil induced G0/G1 phase cell cycle arrest and triggered early apoptosis. GC-MS profiling identified dominant fatty acids, including eicosapentaenoic acid (9.59%), docosahexaenoic acid (10.70%), and 9-octadecenoic acid (9.29%), as well as beneficial saturated fatty acids such as hexadecenoic acid (20.10%). In silico target prediction using SwissTargetPrediction and the Similarity Ensemble Approach (SEA) identified approximately 40-50 potential protein targets linked to CRC, including key regulators such as TP53, PTGS2, NF- κ B, TNF, and NOS2. Gene ontology and KEGG pathway analyses indicated involvement in apoptosis regulation, immune signalling, and PI3K/Akt and p53 pathways. Protein-protein interaction (PPI) analysis highlighted TP53, NF-Kb, and PTGS2 as central hub proteins. Molecular docking simulations demonstrated strong binding affinities (Δ G) below -6.5 kcal/mol both EPA and DHA, suggesting their potential role in modulating key pathways related to cancer progression and immune response. These findings suggest that mackerel scad skin oil can inhibit CRC proliferation and induce apoptosis by targeting crucial molecular regulators. The dual action of marine-derived oil highlights its potential as an anticancer agent and complementary therapeutic candidate for CRC management.



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sciforum-115967: Exploring the Signaling Mechanisms of Kisspeptin Receptor: A Structure-Activity Approach for Designing Safer Therapeutics.

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Introduction

The kisspeptin receptor (KISS1R) is a critical regulator in cancer biology and represents a promising target for therapeutic intervention. Previous studies demonstrated significant antineoplastic activity of novel kisspeptin-10 (KP10) analogs in cervical cancer models. To advance drug development, it is essential to elucidate the specific contributions of KP10 amino acids to receptor signaling and biased agonism.

Methods

A series of KP10 analogs were synthesized, incorporating alanine-scanning mutagenesis (Ala-scan) to systematically replace each amino acid in the sequence. BRET assays, including β -arrestin recruitment and G-proteins activation, were employed to evaluate KISS1R signaling pathways. Selective activation of downstream pathways was analyzed to identify biased agonism profiles.

Results

The Ala-scan revealed key amino acid residues critical for KISS1R activation and pathway-specific signaling. Several analogs displayed biased agonism, preferentially activating G protein or β -arrestin pathways. Notably, analogs with selective signaling profiles demonstrated reduced cytotoxicity while maintaining antineoplastic efficacy in cervical cancer cells. **Conclusions:** This study underscores the importance of specific KP10 residues in modulating KISS1R signaling and highlights the potential for designing biased agonists with enhanced therapeutic profiles. These findings provide a framework for developing safer and more effective drugs targeting KISS1R, minimizing adverse effects while maintaining potent anticancer activity.



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sciforum-123738: Expression of epithelial-mesenchymal transition markers in circulating tumor cells from hepatocellular carcinoma patients after radical resection

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Background

Recurrence of hepatocellular carcinoma (HCC) after radical resection is one of the most important factors that impact the prognosis of patients with HCC, probably due to circulating tumor cells (CTCs) that have been shed from the primary tumor before liver resection. The epithelial-mesenchymal transition (EMT) has a pivotal role in the generation, survival and recolonization of CTCs. CTCs with the EMT phenotype have become recognized as an active source of metastases. In this study, we assessed whether the expression of EMT markers, including Zeb1 and Vimentin in CTCs can predict its recurrence after hepatectomy.

Methods

Peripheral blood circulating tumor cells (CTCs) from 86 hepatocellular carcinoma patients were isolated through a dual-phase enrichment protocol combining Ficoll density gradient centrifugation with immunomagnetic separation. These captured CTCs underwent quadruple immunofluorescence labeling using fluorescent-conjugated anti-CPS1/ASGPR (cellular identification), anti-Zeb1 and anti-Vimentin, along with DAPI nuclear counterstaining, followed by quantitative analysis via fluorescence microscopy within 24 hours preceding hepatectomy. Parallel immunohistochemical evaluation of Zeb1 and Vimentin expression patterns was performed on matched tumor specimens during the perioperative period. Subsequent analytical workflows integrated these molecular profiles with clinical outcomes to elucidate epithelial-mesenchymal plasticity dynamics and their prognostic implications in HCC progression.

Results

CTCs were detected in 87% (75/86) of HCC patients (3-96/5mL). Zeb1/Vimentin phenotypes in CTCs exhibited interpatient heterogeneity, categorized into four subtypes. Zeb1+/Vimentin+ CTCs or CTM correlated with poorest prognosis, independently predicting higher recurrence risk (median RFS: Zeb1+/Vimentin+ ≥ 16 or Zeb1+/Vimentin- ≥ 19 vs lower counts, $p < 0.01$). Tumor tissue Zeb1/Vimentin expression positively correlated with CTC detection.

Conclusions

Zeb1 and Vimentin coexpression may indicate EMT occurs thoroughly. CTCs expressing Zeb1 and Vimentin, suggestive of EMT, are identified in patients with HCC, which may serve as a real-time parameter for monitoring HCC recurrence and pave the way for development of personalized targeted therapy for management of HCC recurrence.



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sciforum-122293: Highly Expressed in Cancer 1 (Hec1): A Novel Therapeutic Target in Human Cancers

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Introduction

Highly expressed in cancer 1 (Hec1) is a key component of the Ndc80 kinetochore complex, which plays a crucial role in mitosis and regulates the spindle checkpoint. Overexpression of Hec1 leads to mitotic checkpoint overactivation and promotes tumorigenesis in transgenic mice. Elevated Hec1 levels have been observed in various cancer cells and tissues, and are strongly associated with poor clinical outcomes in cancer patients. Since phosphorylation of Hec1 by Nek2 kinase is essential for its mitotic function, inhibiting the Nek2–Hec1 protein–protein interaction presents a promising strategy for cancer therapy.

Methods and Results

Over the past decade, we have discovered several potent Hec1 inhibitors derived from the lead compound INH1 through rational drug design. These lead candidates—designated as TAI-1, TAI-2, and TAI-95—exhibited strong anticancer activity at low nanomolar concentrations against a range of cancer cell lines and were also active against multidrug-resistant cancer cells. These compounds bind directly to Hec1 and disrupt the interaction between Nek2 and Hec1, resulting in a reduction in phosphorylated Hec1 and subsequent degradation of Nek2. This disruption induces mitotic catastrophe and apoptosis. When combined with chemotherapeutic agents such as doxorubicin, paclitaxel, or topotecan in selected cancer cell lines, these inhibitors demonstrated synergistic effects. Notably, these compounds showed no activity toward non-cancerous cells, a panel of kinases, or hERG channels—demonstrating cancer specificity, target specificity, and cardiac safety, respectively.

Conclusions

After pharmacokinetic studies, the tosylate salt of TAI-95 was selected for preclinical development and designated as T-1101 tosylate for clinical trials. Phase-I data revealed that T-1101 tosylate exhibited no dose-limiting toxicity. It subsequently received US FDA approval for Phase II clinical trials targeting advanced neuroendocrine tumors. Overall, these findings support the therapeutic potential of Hec1 as a novel target in the treatment of human cancers.



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sciforum-125352: Identification of ANT2 as a Drug-gable Target for Endocrine-Resistant ER α -Positive Breast Cancer

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Introduction: Estrogen receptor (ER) α -positive breast cancer (BC) is commonly treated with anti-estrogen therapy; however, a subset of patients exhibits primary resistance, and many initially responsive cases develop acquired resistance. Overcoming endocrine resistance remains a critical unmet need in the clinical setting of ER α -positive BC. This study aimed to elucidate mechanisms underlying this resistance and to develop therapeutic strategies using a chemoproteoinformatics approach. **Methods:** A natural compound screen based on growth inhibition was performed using ER α -positive BC cells. To identify the molecular target of the compound, it was immobilized on magnetic nanobeads, and target proteins were identified using chemoproteomics. Public database analyses assessed the clinical relevance of the identified target. RNA sequencing was performed on endocrine-resistant BC cells. Molecular dynamics (MD) simulations and *in silico* screening of FDA-approved drug libraries were performed to identify repurposable compounds predicted to bind the target protein. **Results:** Perillyl alcohol (POH), a monoterpene found in herbs such as perilla, was identified as a compound that suppresses ER α expression and cell growth. Chemoproteomics revealed that POH directly binds to adenine nucleotide translocase 2 (ANT2), a mitochondrial inner membrane protein. Public database analyses showed that elevated ANT2 expression correlates with poor prognosis and increased clinical aggressiveness in ER α -positive BC patients. Silencing ANT2 expression phenocopied POH's effects, resulting in ER α downregulation and growth inhibition. POH also suppressed the growth of BC cells resistant to the anti-estrogen agents tamoxifen and fulvestrant. RNA sequencing revealed upregulation of fatty acid elongation-related genes in fulvestrant-resistant cells. Both POH treatment and ANT2 knockdown increased intracellular lipid droplet accumulation, suggesting impaired fatty acid elongation. MD simulations predicted that POH binds to the central pore region of ANT2, which serves as an ATP transport pathway. *In silico* screening identified venetoclax and nystatin as repurposable compounds targeting this site, both of which reduced ER α expression in ER α -positive BC cells and promoted lipid droplet accumulation in fulvestrant-resistant cells. **Conclusions:** Our chemoproteoinformatics approach, integrating chemoproteomics with computational structural biology, highlights ANT2 as a promising druggable target in the pharmacological management of ER α -positive BC, providing a clinically relevant strategy to overcome endocrine resistance by targeting ANT2 with specific ligands.



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sciforum-123255: Infiltration of immunosuppressive myeloid population in hypoxic area of hepatocellular carcinoma determines its response to anti-PD1 therapy

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Hepatocellular carcinoma (HCC) represents a significant public health challenge and is the most prevalent primary liver cancer in adults worldwide. Anti-PD1 therapy is a common treatment for HCC patients, but its effectiveness has shown variable results in recent years. The role of immunosuppressive immune cells in this variability has been noted. In our study, we utilized single-cell spatial transcriptomic analysis to map the distribution of these immunosuppressive cells within the tumor microenvironment of HCC patients who do not respond to anti-PD1 treatment.

Through spatial analysis, we discovered that macrophages and monocytes tend to cluster around the tumor boundary in non-responders. These regions exhibited more enriched immunosuppressive signaling pathways and higher cancer cell proliferation. Hypoxia markers were notably enriched in both responder and non-responder groups, with denser populations of macrophages and monocytes observed in more hypoxic areas, particularly at the interface between fibrotic and carcinoma tissues. In the non-responder group, hypoxic regions correlated with elevated TGF β signaling, ROS, IL6, apoptosis, and angiogenesis expression, while the remaining areas showed high glycolysis expression. These patterns were not seen in the responder group.

In vitro experiments with bone marrow-derived monocytes and macrophages cultured under hypoxic conditions revealed increased immunosuppressive capabilities, including suppression of CD8+ cytotoxic T cell activation and effector function, accompanied by elevated glycolysis. Inhibiting glycolysis in hypoxic myeloid cells reduced their immunosuppressive function. Our findings suggest that the infiltration of immunosuppressive myeloid cells in hypoxic areas of HCC is a key determinant of the response to anti-PD1 therapy.



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sciforum-122420: Inhibition of proliferation of colorectal cancer cells by suppression of claudin-14 expression

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Background and Objective

Mutations in the *KRAS* gene represent one of the most prevalent oncogenic alterations found in various solid tumors. Unfortunately, EGFR-targeting therapies have proven ineffective for patients harboring *KRAS* mutations, necessitating the development of innovative therapeutic strategies. Claudin (CLDN) proteins, which are key cell-cell adhesion molecules, comprise over 20 distinct members. While increased expression of certain CLDN subtypes has been documented in numerous solid tumors, their pathophysiological roles remain largely undefined. So far, we reported that CLDN14 is significantly overexpressed in human colorectal cancer tissues and cell lines. This study aims to elucidate the role of CLDN14 in the proliferation and chemoresistance of colorectal cancer cells.

Methods

We assessed the expression levels of mRNA and protein using real-time PCR and Western blotting, respectively. CLDN14 expression was inhibited through the application of CLDN14-targeting siRNA or PSGMK, a small peptide. The cell cycle was evaluated using propidium iodide staining in conjunction with flow cytometry.

Results and Discussion

The knockdown of CLDN14 expression in human colorectal adenocarcinoma-derived DLD-1 cells, which possess a G12D *KRAS* mutation, resulted in inhibited cell proliferation. Cell cycle analysis revealed a reduction in the S phase population, accompanied by an increase in the G0/G1 phases. Furthermore, CLDN14 silencing led to decreased Rho kinase activity and reduced ERK phosphorylation levels. Similarly, the inhibition of ERK phosphorylation and cell proliferation was observed with Y-27632, a Rho kinase inhibitor, and U0126, a MEK/ERK inhibitor. These findings suggest that CLDN14 promotes cell proliferation through the activation of the Rho kinase/ERK signaling pathway. Additionally, we investigated the effects of PSGMK, a peptide mimicking the second extracellular loop of CLDN14, which also inhibited ERK phosphorylation and cell proliferation. We propose that CLDN14 may serve as a novel therapeutic target for colorectal cancer with *KRAS* mutations.



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sciforum-125459: Ion Channel Profiling and Therapeutic Targeting in Cancer Stem Cells of Digestive Malignancies

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Recent studies have highlighted the therapeutic potential of targeting membrane transporters selectively activated in cancer stem cells (CSCs). This study aimed to characterize ion channel expression profiles specific to digestive CSCs. CSC-enriched populations were isolated from human esophageal squamous cell carcinoma, gastric cancer, and pancreatic cancer cell lines via fluorescence-activated cell sorting based on high expression of established CSC markers, such as ALDH1A1 and CD44. These sorted populations demonstrated enhanced tumorsphere-forming ability and elevated mRNA expression levels of CSC markers compared to non-CSCs, along with increased resistance to conventional chemotherapeutic agents. Microarray analysis revealed distinct upregulation patterns of ion channels: transient receptor potential vanilloid 2 (TRPV2) in esophageal CSCs, voltage-gated calcium channels (VGCCs) in gastric CSCs, and voltage-gated potassium channels (VGKCs) in pancreatic CSCs. Corresponding pharmacologic inhibitors—tranilast for TRPV2, amlodipine and verapamil for VGCCs, and 4-aminopyridine for VGKCs—demonstrated selective cytotoxicity against CSCs over non-CSCs in vitro. These inhibitory effects were further validated in xenograft models using immunodeficient mice. Based on these findings, a phase I/II clinical trial (TNAC study) evaluating the safety and efficacy of neoadjuvant combination chemotherapy with tranilast in patients with advanced esophageal squamous cell carcinoma is currently underway. This research underscores the role of ion channels in CSC maintenance and suggests that their inhibition may represent a promising therapeutic strategy for digestive tract malignancies.



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sciforum-123066: Major Advances and Limitations in CAR T cell Therapy

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An increasing number of blood cancer has led to numerous researches in the now widely experimented chimeric antigen receptor T (CAR T) cell therapy. However, even the last generation of CAR T cells exhibit problems such as limited efficacy for specific types of cancers, lack of antigen specificity, and risks after injection. Here, I review the advances and limitations of CAR T cell therapy and present potential approaches to counteract these limitations, enhancing the efficacy and safety of CAR T cell therapy.

This literature search for this review article was conducted through PubMed and Scopus using the key words: “CAR T cells”, “leukemia”, “immunotherapy”, and more. Studies focusing on the effect of immunotherapy on treating solid tumors and both autologous and allogeneic CAR T cell therapy are included.

T cells derived from PBMCs are able to generate a group of cells that express distinct subtypes of HLA¹. This allows each patient to receive CAR T cells with an HLA that closely matches their own, reducing the risk of rejection. To reduce the risk of GVHD, invariant NK T (iNKT) cells, which are a subset of natural killer (NK) T cells can be used². This is due to their highly cytolytic property to cells that display an appropriate balance of inhibiting versus activating signals. In case of negative effects after injection the inducible caspase-9 (iC9) system is developed. With the presence of CID, the caspase-9 becomes active and induces caspase-3-mediated apoptosis to eliminate the CAR T cells causing adverse effects³. The starting population of mature T cells often includes both CD8+ cytotoxic and CD4+ helper T cells, to maximize the number of these two types of cells, the CD4+ and CD8+ T cells can be isolated separately. However, this manufacturing process requires more labor and cost⁴.

CAR T cell therapy has been successful in clinical trials. However, there are still limitations that require further research to be addressed. Besides minimizing the adverse effects of CAR T cell therapy, the continuous improvement of this therapeutic modality could bring a hopeful future where most kinds of cancer will be treatable.



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sciforum-123270: New insights in the proteolytic contribution of *Ananas comosus* extract to its in vitro anti-cancer effects against colon cancers

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Introduction

Plant cysteine proteinases are enzymes investigated for their therapeutic properties in wound healing, immunomodulation, and as antitumor agents. Our study aimed to investigate their therapeutic potential as complementary agents or sources of new anti-cancer drug candidates through a better understanding of their properties.

Methods

A. comosus raw extract and its purified proteases were prepared and purified by chromatographic techniques. Effects on colon cancer cells were evaluated by viability assays on 2D and 3D spheroid cultures, time-lapse microscopy, adhesion assays, ion homeostasis, and calcium flux assays using a fluorescent fura-2 probe. The relevance of enzymatic activity in vitro was evaluated using the E64 cysteine protease inhibitor.

Results

A. comosus crude extract IC₅₀ from MTT assay on colon cancer cell lines was ~10 µg/mL. Activity was completely lost in presence of the irreversible E64 inhibitor. Time-lapse imaging showed rapid loss of adhesion, confirmed by concentration-response adhesion and detachment assays. Around 50% of cells remained alive for at least 48 hours while already detaching after 3 hours, supporting that adhesion loss occurs prior to cell death. MTT assays with purified acid bromelain, basic bromelain, and ananain enzymes, whose catalytic activities were characterized herein, showed basic bromelain had the strongest effects, followed by ananain, while acid bromelain was inactive. We searched for the Arg-Arg (RR) bromelain cleavage motif in the whole and plasma membrane human proteomes. Functional enrichment of proteins with RR cleavage sites suggests bromelain may affect cell adhesion and monoatomic ion transport. Experimental results support adhesion impairment, which will be further explored on integrin dimers, especially α6β4, considering its 10 potential cleavage sites and its known role in colon cancer aggressiveness. Ion imbalance in basic bromelain-treated cell supernatants and intracellular calcium flux perturbations via Store Operated Calcium Entry also supported theoretical analysis.

Conclusions

Our results show the in vitro anti-cancer effects of *A. comosus* stem proteases are mainly due to basic bromelain's activity on plasma membrane proteins, leading to cell adhesion loss and ion perturbations.



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sciforum-123142: Panobinostat enhances 5-FU efficacy in gastric cancer cells through thymidylate synthase regulation

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Introduction

Gastric cancer remains one of the most prevalent and lethal malignancies worldwide. 5-Fluorouracil (5-FU), a widely utilized chemotherapeutic agent for gastric cancer, exerts its cytotoxic effects by inhibiting thymidylate synthase (TS), an essential enzyme involved in DNA synthesis. However, prolonged administration of 5-FU has been associated with increased TS expression, contributing to acquired drug resistance and reduced therapeutic efficacy. To overcome this limitation, we investigated the molecular regulatory mechanisms of TS expression using panobinostat, an FDA-approved histone deacetylase (HDAC) inhibitor.

Methods

We evaluated the effects of panobinostat, alone and in combination with 5-FU, on cell viability, apoptosis, cell cycle distribution, and TS expression in human gastric cancer cells. Cell viability assays, apoptosis analyses, and cell cycle distribution assays were conducted. TS expression was assessed at both mRNA and protein levels to determine the regulatory effects of panobinostat.

Results

Panobinostat monotherapy significantly decreased cell viability, induced apoptotic cell death, and caused G1 arrest in gastric cancer cells. TS expression was downregulated following panobinostat treatment, suggesting its anticancer effect may be associated with TS regulation. In contrast, 5-FU monotherapy triggered compensatory upregulation of TS, a well-known resistance mechanism. Co-treatment with panobinostat effectively reversed this response, leading to enhanced cytotoxicity and apoptosis compared to either agent alone.

Conclusions

Our findings demonstrate that panobinostat not only exerts direct anticancer effects in gastric cancer cells but also enhances their sensitivity to 5-FU by downregulating TS expression. Furthermore, Panobinostat effectively attenuates 5-FU resistance through HDAC inhibition, presenting a promising strategy for combination therapy in gastric cancer. This research clarifies the molecular mechanism of TS regulation via HDAC inhibition and highlights the potential of combination therapies to bypass chemotherapy resistance. Additionally, our findings underscore the importance of targeting regulatory mechanisms of key enzymes, such as TS, for function-based drug design and the development of precision therapies in future clinical applications.



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sciforum-123138: Sodium butyrate improves 5-fluorouracil sensitivity in gastric cancer via thymidylate synthase and cell cycle regulation

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Introduction

Gastric cancer (GC) is a major cause of cancer-related deaths. 5-fluorouracil (5-FU), a first-line anticancer drug for GC, inhibits thymidylate synthase (TS), a key enzyme in DNA synthesis. However, elevated TS expression contributes to 5-FU resistance, posing a significant clinical challenge. Therefore, targeting TS expression can overcome decreased sensitivity to 5-FU. While several chemicals have been investigated for their effects on TS expression, the role of sodium butyrate (NaB)—a microbiota-derived short-chain fatty acid (SCFA)—remains unclear in GC therapy. In this study, we evaluated if NaB could enhance the sensitivity of GC cells to 5-FU by modulating TS expression and related molecular pathways.

Methods

We utilized AGS (p53 wild-type) and KATO-III (p53-null) gastric cancer cell lines to assess the anti-proliferative effects of NaB alone and in combination with 5-FU. Cell viability assays, spheroid formation assays, apoptosis analyses, and mitochondrial dysfunction assays were performed. Additionally, TS expression at both mRNA and protein levels was measured, along with analysis of key cell cycle regulators to determine the impact of NaB on cell cycle progression.

Results

NaB alone exhibited dose-dependent anti-proliferative effects on GC cells. Co-treatment with NaB and 5-FU significantly reduced cell viability, disrupted spheroid formation, and increased apoptosis and mitochondrial dysfunction compared to 5-FU alone. Notably, NaB treatment suppressed TS expression at both the mRNA and protein levels, accompanied by downregulation of key cell cycle regulators. Furthermore, NaB induced distinct, cell-type specific responses in cell cycle regulation dependent on the p53 status of the cell lines.

Conclusions

Our findings indicate that NaB enhances the efficacy of 5-FU by targeting TS expression and modulating cell cycle regulators in a p53-dependent manner. As a naturally occurring metabolite, NaB may represent a valuable molecular adjuvant capable of overcoming chemoresistance, thereby enhancing the therapeutic potential of 5-FU in gastric cancer treatment.



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sciforum-122541: Sorafenib and regorafenib trigger IL-33-mediated immunosuppression to compromise the treatment efficacy in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is a highly lethal cancer with rising incidence rates globally. The recommended medications for the treatment of advanced-stage HCC are sorafenib and regorafenib; however, acquired resistance to sorafenib and regorafenib treatment significantly limits their therapeutic efficacy, and the underlying mechanisms remain poorly understood. Here, we demonstrated that both sorafenib and regorafenib treatments induce cell senescence in HCC and promote the upregulation and secretion of IL-33 through an IL-33/ST2L positive feedback loop. The secreted IL-33 further enhanced PD-L1 expression in HCC cells by activating NF- κ B pathways in response to sorafenib and regorafenib treatment. Blocking the IL-33 signaling pathway using anti-IL-33 or anti-ST2L neutralizing antibodies, in combination with sorafenib treatment, resulted in a significant reduction in tumor size, growth rate, and weight. Additionally, there was a noticeable decrease in tumor PD-L1 expression and an increase in infiltrating intra-tumor CD8⁺ T cells. Importantly, the enhanced therapeutic efficacy of anti-IL-33 in sorafenib-treated HCC-bearing mice was lost in immunocompromised mice. This indicates that the anti-IL-33 neutralizing antibody enhances the anti-tumor activity of sorafenib by modulating T cell-mediated immunity. Our findings suggest that IL-33 contributes to the diminished therapeutic effectiveness of sorafenib and regorafenib in HCC cells. This study highlights the potential of targeting the IL-33/ST2L axis in combination with targeted therapy as a novel strategy to improve the limited efficacy of sorafenib and regorafenib.



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sciforum-121683: Structure-based drug design for targeting hard-to-target transcription factor STAT3 by focusing on a novel ligand binding site

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Introduction

Signal transducer and activator of transcription 3 (STAT3) has been found for more than three decades, and it plays multiple roles in autoimmune disease, neuropathic pain and cancer. Hence, anti-autoimmune, -neuropathic pain and -cancer drug development focus on targeting STAT3, of which anti-cancer drug development campaigns are the most active. However, no STAT3 inhibitors have been approved for use on the market yet. The SH2 domain and DNA-binding domain (DBD) of STAT3 are the commonly targeted for drug design.

Methods

X-ray crystallography was used to validate binding pocket of STAT3. Cell viability assay was used to determine cytotoxicity of compounds. Western blot was used to investigate indicated protein expression levels. After compound treatment, annexin V/propidium iodide (PI) was used to detect apoptosis and necrosis, while acridine orange staining was used to detect autophagy.

Results

In this study, a novel ligand binding site on STAT3 was validated via co-crystallized with an amide derivative, and the protein structure was resolved using X-ray crystallography. The binding site is neither in the SH2 domain nor DBD, and by focusing on the binding site, a series of novel amide derivatives were designed and synthesized. Using MIA PaCa-2 as model cell line, western blot analysis showed that phosphorylated STAT3 (p-STAT3) levels were significantly inhibited after 24 h treatment with amide derivatives at as low as 100 nM. The upstream kinases of STAT3 signaling, JAK2 and SRC, were not affected by the amide derivatives, while some of the downstream proteins of STAT3 were down-regulated after treatment. Cell viability analysis demonstrated that IC₅₀ values of the amide derivatives ranged from 485 to 7,320 nM after 72 h treatment in pancreatic cancer cell lines MIA PaCa-2, AsPC-1 and Capan-2. Results of annexin V/PI, acridine orange staining and western blot analysis (BCL-2, BAX, caspase 3/9, p62, LC3 protein levels) suggested that the cell death effects of the amide derivatives was autophagy-driven.

Conclusions

Taken together, a new way of inhibiting STAT3 activity by focusing on the novel binding site is validated, and the active, newly developing STAT3 inhibitors warrant further investigation.



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sciforum-123264: Synergistic Antitumor Effects and Underlying Mechanisms of Sodium Butyrate and Dovitinib Co-treatment in A549 Non-Small Cell Lung Cancer Cells

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Background

Lung cancer remains one of the leading causes of cancer-related mortality worldwide, largely due to drug resistance and challenges in early diagnosis. In non-small cell lung cancer (NSCLC), mutations in the epidermal growth factor receptor (EGFR) are predictive of response to tyrosine kinase inhibitors (TKIs). Although EGFR-targeted therapies initially elicit favorable responses, resistance inevitably develops, necessitating new therapeutic strategies. TKIs possess various anticancer properties, including anti-proliferative, anti-inflammatory, and pro-apoptotic effects. Combining TKIs with other agents, such as histone deacetylase inhibitors (HDACis), may enhance therapeutic efficacy through synergistic mechanisms. This study aimed to evaluate the combined antitumor effects of dovitinib (a TKi) and sodium butyrate (NaBu, an HDACi) in A549 human NSCLC cells, with a focus on oxidative stress, apoptosis, and related signaling pathways.

Methods

A549 cells were treated with dovitinib (20 μ M), sodium butyrate (20 mM), or their combination for 18 and 24 hours. Flow cytometry was employed to assess apoptosis, cell cycle progression, and oxidative stress. Mitochondrial membrane potential (MMP) was evaluated using JC-1 staining. Western blot analysis was performed to examine protein expression related to apoptosis, autophagy, and inflammation, while apoptotic gene expression was assessed via qPCR.

Results

Combined treatment with dovitinib and NaBu significantly increased apoptotic cell populations and oxidative stress levels compared to either agent alone. The combination therapy also upregulated the expression of cleaved caspase-3 and LC3B-II/I, while downregulating Cyclin D1, TAZ, NF- κ B, and STAT3.

Conclusion

Taken together, these data suggest that the combination of dovitinib and NaBu effectively enhances apoptosis and autophagy in human lung cancer cells while reducing inflammation. These findings suggest that the combination of dovitinib and NaBu exerts synergistic antitumor effects in A549 NSCLC cells by enhancing apoptosis and autophagy while suppressing inflammatory signaling pathways. This combinatorial approach may offer a promising strategy for overcoming TKi resistance in NSCLC therapy.



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sciforum-126231: The circ_0071616-miR-140-3p-USP34 axis mediates FoxM1 deubiquitination in Helicobacter pylori-induced gastric malignant transformation

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Helicobacter pylori infection can induce malignant transformation of gastric mucosal epithelial cells, and the role of "epigenetic reprogramming" involving environmental factors, abnormal expression heterogeneity of host genes, and regulation of tumor microenvironment in this process deserves attention. Both high-throughput sequencing and bioinformatics analysis results suggested that the pathway involved key nodes circ_0071616 / miR-140-3p / USP34 could affect cell proliferation and ubiquitin-related signal transduction in helicobacter pylori infection-related gastric malignant transformation. The results from qRT-PCR, Western blotting, EdU assay, CCK-8 assay, colony formation assay, SA- β -gal staining, and subcutaneous tumor formation experiment in nude mice demonstrated that circ_0071616 could promote gastric carcinogenesis by enhancing cell proliferation and inhibiting cell senescence. Online database prediction results along with luciferase activity detection, FISH experiment findings and correlation analysis of genes expression confirmed the direct binding between circ_0071616 and miR-140-3p as well as their functional interaction. TCGA database screening combined with cellular molecular experiments and animal model studies further validated miR-140-3p as a tumor suppressor in gastric cancer by inhibiting cell proliferation while promoting cell senescence. Subsequent investigations revealed that circ_0071616 regulated miR-140-p to directly bind to the core sequence within USP34 3'-UTR region leading to inhibition of the oncogenic molecule deubiquitinating enzyme USP34 expression thereby stabilizing FoxM1 protein levels which ultimately contributes to the gastric carcinogenesis and development. This study provides preliminary evidence for the mechanism of Helicobacter pylori infection on the deubiquitination process of FoxM1, a crucial positive regulatory protein in cell cycle regulation, through the circ_0071616 - miR-140-3p - USP34 pathway. These findings can offer novel insights into the early diagnosis and treatment strategies for gastric cancer.



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sciforum-122991: The Dynamic Feedback Between IL-6 and TWIST1 Drives Pancreatic Cancer Progression

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Background

Interleukin-6 (IL-6) and twist family bHLH transcription factor 1 (TWIST1) are implicated in cancer progression. In this study, we explored the relationship between TWIST1 and IL-6 in pancreatic ductal adenocarcinoma (PDAC), highlighting the feedback loop formed by TWIST1 regulating IL-6 expression, which in turn influences miRNA-543 (miR-543) to target TWIST1.

Methods

The levels of IL-6, IL-6R, and TWIST1 was analyzed in 66 PDAC patient samples using tissue microarrays, and their associations with clinicopathological parameters and overall survival were evaluated. miR-543 was selected that could regulate TWIST1. The IL-6/miR-543/TWIST1 pathway was confirmed in PDAC cell lines through quantitative reverse transcription PCR, western blotting, immunofluorescence, enzyme-linked immunosorbent assays, and dual-luciferase reporter assays. Chromatin immunoprecipitation identified the feedback loop between TWIST1 and IL-6. The biological functions of the IL-6/miR-543/TWIST1 axis in PDAC were further investigated through *in vitro* and *in vivo* experiments. Furthermore, Tocilizumab, an anti-IL-6 receptor monoclonal antibody, was shown to disrupt the IL-6/miR-543/TWIST1 circuit, thereby suppressing PDAC progression.

Results

IL-6, IL-6R and TWIST1 were elevated in human PDAC tissue and correlated with unfavorable prognosis. A meaningful association between IL-6 and TWIST1 was verified. Based on publicly available algorithms, miR-543 was predicted, selected and demonstrated as the regulatory microRNA between IL-6 and TWIST1. Then, *in vitro* and *in vivo* tests showed that IL-6 not only modulates TWIST1 expression via miR-543, but also influences the migration, invasion, proliferation, and colony formation of PDAC cells, as well as tumor growth, through the same signaling pathway. Moreover, miR-543 could reverse the IL-6 effects on the PDAC cells *in vitro* and *in vivo*. Interestingly, TWIST1 was found to interact with the IL-6 promoter, promoting the expression and secretion of IL-6. Therefore, a positive feedback loop between IL-6 and TWIST1 was found, and interference with this loop suppressed the progression of PDAC.

Conclusion

Our findings confirm that the IL-6/miR-543/TWIST1 feedback loop plays a crucial role in the malignant biological behaviors of PDAC, with TWIST1 acting as a key regulator of IL-6 expression.



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sciforum-123334: The potential of ion channels as therapeutic targets in gastrointestinal cancers

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Ion channels have been recently shown to be involved in cellular functions of various cancers, and many kinds of channel types have been detected in gastrointestinal cancers. Our laboratory have focused on the expression and role of ion channels and investigated the potential of their regulation for cancer management.

We have previously conducted *in-vitro* and *in-vivo* experiments using cancer cells to assess cellular physiological functions of ion channels, especially Cl⁻ and Ca²⁺ channels, in various gastrointestinal cancers. We have shown that the pharmacological blockage and gene silencing against these channels had an impact on carcinogenesis. We have also demonstrated an immunohistochemical staining to assess the protein expression level of each ion channel using clinical samples and reported about the clinicopathological and prognostic significance of their expression in various gastrointestinal cancers.

Furthermore, we have also reported that the blockage of Cl⁻ channels enhanced the cytotoxic effects of hypotonic shock by inhibiting regulatory volume decrease in various gastrointestinal cancers, which supported the efficacy of hypotonic treatment combined with ion channel blockers against cancer pleural or peritoneal dissemination.

These results indicate that the regulations of ion channels have potential as a novel biomarker and also an effective treatment strategy by cellular physiological approaches in gastrointestinal cancers.



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sciforum-125440: Using Adhiron technology to develop PAK1-selective inhibitors for targeted cancer treatment

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Cancer remains a leading cause of death worldwide, with the dysregulation of the cell cycle being a hallmark of all human cancers. p21-activated kinase 1 (PAK1) plays a crucial role in cell cycle regulation and is often overexpressed in certain cancers. Due to its involvement in multiple oncogenic pathways, PAK1 has emerged as a promising therapeutic target. However, as a member of the PAK family, PAK1 has distinct as well as overlapping functions and structures with other isoforms, making selective targeting challenging. This study explores the use of non-antibody engineered proteins, known as Adhiron, to selectively inhibit PAK1, highlighting their potential for novel cancer treatment strategies.

Using phage display and high-throughput screening, three Adhiron (P60, P66, and P73) were identified with inhibitory effects on PAK1, exhibiting inhibition, 99.74%, 91.19%, and 35.7%, respectively. Structural studies revealed distinct modes of inhibition among Adhiron. P60 functions as an ATP-competitive inhibitor, occupying the ATP-binding site of PAK1, whilst P66 and P73 act as an allosteric inhibitor, binding away from ATP-binding pocket. Cross-reactivity and surface plasmon resonance studies showed, 1) P66 exhibited high affinity and broad binding across the PAK family, 2) P60 showed also high affinity but interacted with PAK1, PAK2, and PAK3, and 3) P73 showed lowest affinity but had highest specificity for PAK1. Differences in PAK1 inhibitory and specificity activities were related to variations in Adhiron-PAK1 interaction and conformational effects on each PAK.

Our strategy has enabled the successful identification of PAK1 inhibiting Adhiron which will offer valuable insights for the development of PAK1 specific small molecules.



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Session 5. Drug Design and Solution to Drug Resistance Problem

sciforum-123032: Design and Development Strategy for Target Proteins used in Antibody Drug Discovery

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ACROBIOSYSTEMS CO.,LTD.

Introduction

Target proteins play a pivotal role in antibody drug discovery, serving as critical reagents for immunization, screening, and validation of therapeutic antibodies. Their structural integrity and functional activity are essential for generating high-affinity antibodies and ensuring accurate epitope presentation. This research outlines a comprehensive strategy for the design, development, and characterization of target proteins, emphasizing the integration of structural analysis and application-specific requirements.

Methods and Materials

Key components of recombinant protein development include expression system optimization (e.g., codon usage, host cells), rigorous quality control and advanced technologies such as HEK293-based platforms for difficult-to-express proteins and nanoparticle antigen displays. Computational modeling based on the targeted protein sequence was performed to predict the disorder along with interface and interaction region statistics. Chromatography using a SEC column was used to analyze protein aggregation, and SPR/BLI/ELISA studies are used to evaluate protein-protein interactions and ensure proper binding.

Results

Case studies highlight the importance of native conformation, as demonstrated by RSV F glycoprotein's pre- and post-fusion states, and the enhanced bioactivity of homogenous trimeric BAFF. Additionally, dimeric CTLA-4 exhibits a 10-fold higher affinity than its monomeric form, underscoring the impact of native oligomerization on drug screening. Varying protein formats are also analyzed based on its platform, ensuring uniform size, structure, and bioactivity for each protein.

Conclusion

By addressing challenges such as aggregation and conformational stability, these strategies ensure the production of high-quality target proteins, ultimately advancing the discovery of potent and specific therapeutic antibodies.



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sciforum-123302: Deubiquitinating enzymes associated with paclitaxel response in lung cancer cells

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Introduction

Lung cancer is one of the leading causes of cancer-related deaths worldwide and is often diagnosed at advanced stages. Non-small cell lung cancer (NSCLC), which accounts for approximately 85% of all lung cancer cases, is commonly treated with paclitaxel (PTX). However, the emergence of resistance to PTX severely hampers its clinical efficacy, highlighting the urgent need for novel therapeutic strategies. The ubiquitin-proteasome system (UPS), which regulates protein degradation, plays a pivotal role in cancer progression and drug resistance. Among its components, deubiquitinating enzymes (DUBs) have emerged as potential modulators of chemotherapy sensitivity.

Methods

To identify *DUB* genes associated with PTX responsiveness, we treated A549 cells with PTX and conducted a multiplex RT-PCR analysis to assess changes in the expression of these genes. The expression of candidate DUB proteins was further validated by western blotting using A549 and H1299 cells. The UALCAN website, utilizing data from The Cancer Genome Atlas (TCGA) database, was used to analyze the expression levels of *DUBs* in lung adenocarcinoma (LUAD). The GEPIA database (Gene Expression Profiling Interactive Analysis) was employed to generate overall survival curves for patients with LUAD and lung squamous cell carcinoma (LUSC).

Results

Our screening results showed that the expression of several *DUB* genes, including *PTX-DUB1*, *-DUB2*, *PTX-DUB3*, *PTX-DUB4*, *PTX-DUB5*, and *PTX-DUB6*, changed in response to PTX treatment. *PTX-DUB1*, *PTX-DUB2*, *PTX-DUB4*, and *PTX-DUB5* showed significantly reduced mRNA levels in A549 cells, consistent with protein expression. In contrast, *PTX-DUB3* and *PTX-DUB6* showed increased mRNA in A549 cells, with *PTX-DUB3* also upregulated at the protein level. Notably, *PTX-DUB6* showed a cell line-specific decrease in protein in H1299 cells. When comparing *DUB* mRNA levels between normal and lung cancer patients, most were elevated in cancer, except for *PTX-DUB1* and *PTX-DUB5*. Survival analysis showed that, aside from *PTX-DUB3*, low DUB expression was not clearly linked to better prognosis. *PTX-DUB3* may serve as a potential prognostic marker in lung cancer.

Conclusion

Our findings suggest that specific DUBs may influence the cellular response to PTX in NSCLC cells. Modulating their expression could provide a novel strategy to overcome PTX resistance, ultimately improving therapeutic outcomes for lung cancer patients.



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sciforum-126491: In Silico Analysis of Antibacterial Phenylphenalenones from *Musa textilis* (Abaca) Pseudostem: A Molecular Docking Approach

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The escalating threat of antibiotic-resistant pathogens has intensified the pursuit of novel antimicrobial agents derived from sustainable natural sources. This study evaluated the antibacterial potential of eight phenylphenalenones, putative compounds from *Musa textilis* (abaca) pseudostem by targeting six clinically-important bacteria with reported drug resistance: *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Protein structures were screened by selecting only high-resolution X-ray diffraction models retrieved from RCSB Protein Data Bank, while the receptor proteins underwent complete optimization using AutoDock Tools (ADT) by removing heteroatoms, adding polar hydrogens, and applying Kollman charges. The ligand structures of phenylphenalenones (Quercetin 3-o-rutinoside, Quercetin, p-Coumaric acid, Kaempferol, Ferulic acid, Chlorogenic acid, Caffeic acids, and Apigenin) were geometrically optimized by following the drug-likeness parameters guided by Lipinski's Rule of Five. Molecular docking was facilitated using AutoDock Vina, ensuring that grid boxes were aligned within the active site residues. Heat map visualization of the binding affinities coupled with Ramachandran plots confirmed the geometrical suitability and structural integrity of protein-ligand complexes. Using ChimeraX, the interaction profiles were then rendered to identify hydrogen bonds and lipophilic interactions at major binding pockets. Binding affinity scores ranged from -5.38 to -8.81 kcal/mol, wherein the strongest binding was observed in p-Coumaric acid binding with *S. aureus*. Whereas Quercetin garnered the highest mean docking score of -7.95 kcal/mol across multiple bacterial targets. Two-way ANOVA revealed significant differences ($p < 0.05$) between phenylphenalenone interactions and Levofloxacin as control ligand, against *A. baumannii* ($p = 0.08$), suggesting selective inhibition. In conclusion, *Musa textilis*-derived phenylphenalenones exhibited overall antibacterial capability, as emphasized in post-molecular docking analysis, serving as a robust tool to optimize putative compounds. Furthermore, in-vitro and in-vivo studies are warranted to validate the bioavailability of phenylphenalenones.



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sciforum-122796: The crosstalk between Ubiquitination and GlcNAcylation of CHAF1A regulates the HIV-1 latency and reactivation

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Abstract

The persistence of latent HIV-1 reservoirs remains a critical barrier to cure. Current "shock and kill" strategies are limited by ineffective latency-reversing agents (LRAs) and poor understanding of epigenetic regulation. Here, we identify chromatin assembly factor 1 subunit A (CHAF1A), a histone chaperone enforcing HIV-1 latency, as a therapeutic target regulated by antagonistic post-translational modifications (PTMs): ubiquitination promotes its degradation, while O-GlcNAcylation stabilizes it. We demonstrate that trifluridine, an FDA-approved antiviral drug, reactivates latent HIV-1 by disrupting O-GlcNAcylation, triggering CHAF1A ubiquitination and proteasomal degradation. Notably, CHAF1A expression increases with age in CD4⁺ T cells (>60 years), correlating with deeper proviral reservoirs. This age-dependent accumulation inversely associates with reduced O-GlcNAcase (OGA) levels, suggesting O-GlcNAcylation-mediated stabilization in aging. Our findings establish CHAF1A as both a therapeutic target and an age-stratifying biomarker, advancing trifluridine as a translatable LRA to enhance reservoir clearance in aging populations—a demographic increasingly impacted by HIV-1 persistence.

Importance

HIV-1 latency continues to represent a significant barrier to achieving a cure, particularly in aging populations characterized by expanded viral reservoirs and compromised immune recovery—a challenge further intensified by the absence of therapies specifically designed to target age-related mechanisms. Current LRAs are insufficient in addressing the metabolic and epigenetic dysregulation that sustains viral persistence in older individuals. In this study, we reveal a dynamic interplay between ubiquitination and O-GlcNAcylation that regulates the stability of CHAF1A, a histone chaperone essential for maintaining HIV-1 latency. We identify trifluridine as a novel LRA capable of disrupting O-GlcNAcylation to degrade CHAF1A, thereby effectively reversing latency in primary cells. This research bridges a critical gap between fundamental virology and clinical gerontology. These findings establish a robust foundation for refining strategies aimed at HIV-1 eradication, with a focus on targeting host metabolic-epigenetic networks to address latency in underserved aging populations.



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sciforum-123073: Attenuation of Alzheimer's Disease-Associated Neuroinflammation Using Microglia-targeting Luteolin Nanoliposomes: A Proof-of-Concept Study

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the amyloid beta (A β) plaques and tau pathology. Impaired mitophagy, the selective degradation of damaged mitochondria, has been implicated in AD pathogenesis. This study investigates the role of mitophagy impairment in AD development and evaluates the therapeutic potential of luteolin, a natural compound, delivered via microglia-targeted nanoliposomes to enhance mitophagy and reduce neuroinflammation.

Method

We generated a mitophagy-reporting mouse model, Mito-QC-5xFAD, by crossing 5xFAD mice with Mito-QC mice to monitor mitophagy dynamics across pathological stages. Microglia-specific nanoliposomes loaded with luteolin were developed using thin film dispersion to enhance targeted delivery. PLX3397 was administered to deplete microglia and assess their role in luteolin's therapeutic effects. Mitophagy levels, neuroinflammation, and cognitive function were evaluated using Western blotting, flow cytometry, immunohistochemistry, and Morris water maze tasks.

Results

Mitophagy impairment was detected in 2-month-old Mito-QC-5xFAD mice, preceding A β plaque accumulation. Luteolin treatment significantly reduced neuroinflammation, promoted microglia polarization towards a disease-associated (DAM) state, and improved spatial memory in the Morris water maze. These effects were abolished upon microglia depletion with PLX3397, correlating with increased A β plaque burden. Luteolin nanoliposomes were successfully formulated with a uniform size of ~100 nm, enhancing drug delivery efficiency.

Conclusion

Early mitophagy impairment in microglia contributes to the AD pathogenesis, and luteolin acts as a potent mitophagy activator, alleviating neuroinflammation and improving cognitive function through microglial modulation. These findings highlight the therapeutic potential of luteolin nanoliposomes as a targeted strategy for AD intervention.

Acknowledgement

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sciforum-119998: Characterization and Therapeutic Potential of Au-Col-Allicin Nanocomposite in DBTRG Glioblastoma Cells

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This study comprehensively characterizes the Au-Col-Allicin nanocomposite as a potential drug carrier and evaluates its therapeutic efficacy against DBTRG glioblastoma cells. UV-Vis spectroscopy confirmed the presence of gold nanoparticles (AuNPs) with a characteristic peak at 520 nm, while FTIR analysis identified key functional groups, confirming the successful integration of collagen and allicin within the nanocomposite. SEM and DLS measurements revealed a substantial increase in particle size upon conjugation with collagen and allicin, indicating efficient allicin loading. Zeta potential analysis demonstrated shifts in surface charge, suggesting enhanced interaction with target cells and improved cellular uptake. Cytotoxicity and cell viability assays revealed selective toxicity towards DBTRG cells, with minimal impact on bovine aortic endothelial cells. Cellular uptake studies using fluorescence microscopy and FACS confirmed significant internalization and lysosomal targeting of Au-Col and Au-Col-Allicin in DBTRG cells, facilitating intracellular transportation and retention. Apoptosis assays indicated a marked increase in the sub-G1 population and Annexin V-positive cells upon Au-Col-Allicin treatment, confirming apoptosis induction. Additionally, migration inhibition assays demonstrated that Au-Col-Allicin significantly reduced DBTRG cell migration and suppressed MMP-2 activity, highlighting its anti-migratory properties. Furthermore, Au-Col-Allicin effectively mitigated reactive oxygen species (ROS) production in DBTRG cells, underscoring its antioxidant potential. ELISA assays revealed substantial reductions in pro-inflammatory cytokines TNF- α and IL-1 β , indicating anti-inflammatory activity, though its effect on IL-6 was not significant. Collectively, these findings highlight Au-Col-Allicin as a promising nanocomposite with targeted cytotoxicity, efficient cellular uptake, apoptosis induction, anti-migratory effects, antioxidant capacity, and anti-inflammatory properties, supporting its potential application in glioblastoma therapy.



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sciforum-127434: Comparative Docking and Systems-Level Terpenoid Integration Show Celastrol's Multi-Target Modulator in Parkinson's Pathophysiology

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The neurodegenerative condition of Parkinson's disease (PD) includes multiple pathogenic elements, such as dopaminergic neuronal loss and mitochondrial abnormalities, with protein clumping and reactive oxygen species damage. Our research examined potential disease-modifying drugs, for non-symptomatic treatment. To this end, we carried out molecular docking studies and systems-level analysis of four lesser-studied plant-derived terpenoids, including Celastrol, Ginkgolide J, Andrographolide and Caesalpin A and compared results with Levodopa. We mainly evaluated their binding affinity to four crucial PD-related interconnected proteins (STRING-based protein-protein interconnection mapping), including PINK1, Parkin (PRKN), DJ-1 (PARK7), and alpha-synuclein (SNCA).

AutoDock Vina docking studies showed all terpenoids had greater binding affinities than those of Levodopa to all target proteins (−4.3 and −6.9 kcal/mol). Celastrol demonstrated binding affinity to PINK1 at −10.6 kcal/mol and to PARK7 at −7.4 kcal/mol. Ginkgolide J had affinity to PINK1 at −9.3 kcal/mol and to PRKN at −8.2 kcal/mol. STITCH analysis evidenced that Celastrol interacts with cellular stress regulators such as HSP90AA1, MAPK8, TNF, VEGFA and HMOX1, pointing to its involvement in both inflammatory processes and proteostasis control.

KEGG analysis demonstrated that PINK1, PRKN, PARK7, SNCA significantly increase in the Parkinson's disease pathway (hsa05012; FDR = 7×10^{-6}) and mitophagy one (hsa04137; FDR = 0.0111). The strong docking interactions of Celastrol with PINK1, PRKN, and PARK7 and its influence on protein homeostasis make it a viable scaffold for restoring mitochondrial function and redox equilibrium.

Despite not modified terpenoids face restrictions in blood-brain barrier penetration, Celastrol, upon additional optimisation to address this issue, could serve as a disease-modifying agent for ameliorating Parkinson's disease condition.



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sciforum-126280: Development and evaluation of novel dual-target drugs against visceral leishmaniasis

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Visceral Leishmaniasis (VL) is a parasitic protozoan disease infecting human hosts, with high-rate mortality if not treated. So, there is an urgent need for the development of novel treatments due to the limitations of currently available drugs. Two dual-target inhibitors (ZINC000008876351 and ZINC000253403245) were identified by utilizing an advanced computational drug discovery method, both capable to target and inhibit two critical enzymes such as FeSODA (Iron superoxide dismutase) and TryR (Trypanothione reductase), within the antioxidant defence system of *Leishmania donovani* (Ld). *In vitro* enzyme inhibition kinetics were performed observing K_i in the low micromolar range for both drugs. Further assessment of the conformational stability of the inhibitor-protein interactions was conducted using circular dichroism spectroscopy, providing strong insight into their binding properties. *In vitro* cell-based assay exhibited strong anti-leishmanial activity in a dose-dependent manner against both the promastigote and amastigote stages of the parasite. Notably, both these drugs induced ROS overproduction at IC_{50} and $2X IC_{50}$ concentration and caused apoptosis-like cell death, as evidenced by mitochondrial membrane depolarization and phosphatidylserine externalization. Additionally, when both drugs were combined with standard anti-leishmanial drug miltefosine, a synergistic effect was observed, thus making these drugs attractive against *L. donovani* possible resistance. Overall, our results demonstrated that the novel dual-target drugs ZINC000253403245 and ZINC000008876351 are promising future candidates for the development of innovative chemotherapeutic agents for the treatment of visceral leishmaniasis, paving the way for improved therapeutic strategies against this disease.



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sciforum-123294: Differential expression of deubiquitinating enzymes in cisplatin-treated lung cancer cells

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Introduction

Lung cancer is a highly lethal disease characterized by a significant mortality rate. Cisplatin, a common drug used for lung cancer treatment, frequently develops resistance over time. Therefore, overcoming cisplatin resistance is crucial in the effective management of lung cancer. The ubiquitin-proteasome system (UPS) serves as a vital regulatory mechanism for maintaining protein homeostasis within cells. Recent studies have shown that manipulating deubiquitinating enzymes (DUBs) can overcome cisplatin resistance. This study aims to investigate the expression levels of DUBs under cisplatin treatment.

Methods

Multiplex RT-PCR analysis was performed to identify potential biomarkers by comparing the differential expression patterns of *DUBs*, and their expression levels were analyzed by RT-qPCR. In addition, their protein expression levels were determined by western blot analysis. The bioinformatics tools including TCGA database and GEPIA website were used to validate potential as prognostic markers in lung cancer.

Results

Multiplex RT-PCR analysis demonstrated differences in the mRNA expression levels of several *DUBs*, including *DUB7*, *DUB49*, *DUB56*, *DUB60*, *DUB77*, and *DUB82*, in lung cancer cells treated with cisplatin. In addition, RT-qPCR analysis revealed that the expression levels of *DUB49*, *DUB56*, *DUB60*, *DUB77*, and *DUB82* were diminished by approximately 0.64-fold, 0.56-fold, 0.72-fold, 0.66-fold, and 0.68-fold, respectively, while *DUB7* elevated by approximately 1.72-fold under cisplatin treatment. The protein expression level of DUBs was examined by western blotting analysis with increasing concentrations of cisplatin, which was consistent with the trends in the mRNA expression levels, except for *DUB77*, which showed no significant change in expression. To further assess the potential value as biomarkers, we compared the expression levels and overall survival in lung cancer based on TCGA database. Our analysis identified an upregulation of *DUB49*, *DUB56*, *DUB60*, *DUB77*, and *DUB82*, whereas *DUB7* exhibited downregulation in lung cancer patients. In addition, with the exception of *DUB56* and *DUB60*, lower expression levels of the identified *DUBs* were associated with increased survival rates.

Conclusion

These findings strongly suggest that DUBs may play a crucial role in overcoming cisplatin resistance and improving the treatment efficacy for lung cancer.



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sciforum-122751: Discovery Of Moracin D As A Novel Interleukin-1 β Inhibitor To Alleviate Acute Lung Injury

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Target

Inhibition of interleukin-1 β (IL-1 β) is recognized as one of the effective strategies for anti-inflammatory therapy. However, current IL-1 β inhibitors are protein-based products, which are costly and require stringent storage conditions. Therefore, orally available natural compounds that inhibit IL-1 β hold promise as potential therapeutic agents.

Method

In our previous work, we utilized molecular screening and dynamic simulation to discover that Moracin D can stably bind to the active pocket of IL-1 β . In this study, we employed Microscale Thermophoresis (MST) experiments to validate the binding affinity of Moracin D to IL-1 β and further confirmed the binding affinity of IL-1 β complexed with Moracin D to its receptor. We employed Limited proteolysis-coupled mass spectrometry analysis to identify the peptide segments of IL-1 β that interact with Moracin D. In vitro cellular experiments were conducted to verify the action and molecular mechanisms of Moracin D in blocking IL-1 β . Finally, an acute lung injury model induced by LPS was utilized to confirm the anti-inflammatory effects and safety of Moracin D.

Result

We found that Moracin D can tightly bind to IL-1 β with an association constant of 0.1 μ M through MST experiments, and the binding affinity of IL-1 β complexed with Moracin D to its receptor is reduced. We confirmed the key amino acid residue of IL-1 β involved in the interaction with Moracin D using Lip-MS technology, which was identified as ILE56 residue. IL-1 β complexed with Moracin D exhibited reduced ability to upregulate IL-6 and IL-8 in vitro. Meanwhile Moracin D was found to inhibit the phosphorylation of Focal Adhesion Kinase in macrophages, thereby reducing the production of IL-1 β in macrophages stimulated by LPS. Oral administration of Moracin D to mice with LPS-induced acute lung injury for 36 hours resulted in a decrease in pulmonary inflammatory cytokines (IL-1 β , IL-6 and TNF- α) (Figure 2A). Hematoxylin and eosin staining showed a reduction in neutrophils within lung tissue, and a significant alleviation of pulmonary edema and interstitial hemorrhage (Figure 2B), without causing damage to liver or kidney function.

Conclusion

These findings identify Moracin D as a novel oral IL-1 β inhibitor with promising therapeutic potential for ALI associated with inflammation.



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sciforum-125696: Drug design for the solution to drug resistance

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The appearance of drug resistance among infectious agents is a serious medical problem. Resistance makes infectious diseases untreatable. Drug resistance is also a central problem of cancer treatment. Since the resistance originates from the revolution capability of living organisms, its appearance is inevitable. It occurs by mutation of the drug target or efflux, or by acquisition of drug-disrupting activity. I reviewed several developments to circumvent this problem. Rational and quick drug design methods are well developed with the advancement of AI-based and structure-based approaches. Chemical synthesis of new drugs, a limiting step of drug development, is accelerated by combinatorial methods. Peptides and oligonucleotides (aptamers) are used as drugs to avoid complicated chemical synthesis. Monoclonal antibodies are produced by a biological process and used as drugs. The synthesis of undesired proteins can be prevented by designing oligonucleotides (nucleic acid drugs) binding to the gene or mRNA. The design of this type of drug does not require structural analysis of the target and is easily adapted to the mutants. The drug uptake and secretion mechanisms begin to be understood by biochemical analyses of transport systems. Inhibition of homologous recombination was proposed to avoid propagation of resistance genes among bacteria through horizontal transfer or the appearance of cancer variants.



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sciforum-122461: Formulation of *Salvia dumetorum* Extract-Based Tablets for the Treatment of *Streptococcus mutans* Infections: Antimicrobial and Antibiofilm Properties

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In response to the global threat of antimicrobial resistance, this study investigates *Salvia dumetorum* extract as a potential active ingredient for developing antibiofilm agents against *Streptococcus mutans*, a major cause of dental caries. *S. mutans* forms biofilms on tooth surfaces that resist conventional antibiotics, complicating the treatment of oral infections. The study focuses on formulating tablets containing *Salvia dumetorum* extract to address these infections by inhibiting biofilm formation and disrupting pre-formed biofilms.

Tablet formulations were developed using an ethanol extract of *Salvia dumetorum*, incorporating varying extract concentrations through granulation, compression, and coating techniques. The tablets were tested for stability, uniformity, and controlled release. Their antimicrobial and antibiofilm efficacy was evaluated in vitro using *S. mutans* cultures, with a crystal violet colorimetric assay to measure biofilm inhibition. The minimum inhibitory concentration (MIC) and minimum biofilm inhibitory concentration (MBIC) were also determined.

The tablets containing *Salvia dumetorum* extract demonstrated effective antimicrobial activity, significantly reducing biofilm formation in a dose-dependent manner and disrupting pre-formed biofilms. The release studies showed a controlled release of the active compounds, ensuring sustained antibacterial and antibiofilm effects. The MIC and MBIC values aligned with those observed for the extract alone, confirming the tablets' efficacy in combating *S. mutans*.

These findings suggest that tablets formulated with *Salvia dumetorum* extract are a promising therapeutic solution for *S. mutans*-related oral infections, offering both antimicrobial and antibiofilm activities. Further clinical studies are needed to assess the long-term efficacy, safety, and patient acceptability of these formulations in real-world applications.



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sciforum-123018: IL-15 Blocks Autophagic Flux in Pancreatic Stellate Cells to Enhance Alanine Secretion and Sustain Pancreatic Cancer Gemcitabine Resistance

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) poses formidable therapeutic challenges due to its high resistance to the first-line drug gemcitabine (GEM). While existing studies have focused on tumor-intrinsic mechanisms or stromal barriers, the dynamic metabolic interplay between drug-resistant cells and pancreatic stellate cells (PSCs) within the tumor microenvironment (TME) remains poorly understood.

Objectives

This study aimed to elucidate how GEM-resistant PDAC cells orchestrate metabolic crosstalk with PSCs through interleukin-15 (IL-15)-mediated regulation of autophagic flux and alanine (Ala) secretion, ultimately establishing a GEM-resistance feedback loop.

Methods & Results

Cytokine microarray analysis showed that GEM-resistant cells highly expressed interleukin-15 (IL-15). Furthermore, using a combination of an *in vitro* cell co-culture model, transcriptome sequencing analysis, and an *in vivo* orthotopic xenograft model, we sought to elucidate the role of IL-15 from GEM-resistant cancer cells in regulating FOS expression in PSCs. Mechanistically, IL-15 from GEM-resistant cells suppresses autophagy flux in PSCs and promotes their secretion of Ala into the TME. This PSCs-derived Ala establishes a GEM resistance-positive feedback loop by enhancing PDAC cell survival under GEM treatment. Notably, we confirmed that IL-15 inhibitor AMG714 enhances the sensitivity of PDAC to GEM *in vivo*.

Conclusion

Our findings unveil a stromal-epithelial metabolic axis driven by GEM-resistant cells as activators, which modulates PSCs autophagy and Ala secretion to sustain GEM-resistant PDAC, and identified IL-15 as a potential therapeutic target for GEM-resistant PDAC.



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sciforum-119847: Integrated Computational Approaches Identified Gallic Acid–Flavonoid Conjugates as Effective Inhibitors against SARS-CoV-2 Main Protease (M^{pro})

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The COVID-19 pandemic, triggered by the SARS-CoV-2 in 2019, has presented a significant global public health crisis that continues to evolve. A key target for the development of new antiviral agents is the SARS-CoV-2 main protease (M^{pro} or 3CL^{pro}), which plays a crucial role in viral replication by cleaving viral polyproteins into the functional subunits necessary for viral assembly and maturation. In this study, fragment molecular orbital (FMO) calculations were employed to investigate protein-ligand interactions between SARS-CoV-2 M^{pro} and flavonoid inhibitors using four available crystal structures. These calculations guided the structural modification of natural flavonoids, leading to the identification of the galloyl moiety as a critical component for enhanced binding. Two flavonoids from *Boesenbergia rotunda* (aka fingerroot), pinostrobin and pinocembrin, were conjugated with gallic acid *via* esterification. An *in vitro* enzymatic assay demonstrated that these galloyl-flavonoids exhibited significantly enhanced anti-proteolytic activity—up to 23-fold higher than their parent compounds. Notably, 7-*O*-galloylpinocembrin displayed an IC₅₀ value of 8.78 μM against the wild-type SARS-CoV-2 M^{pro}. Additionally, three independent 300 ns molecular dynamics simulations provided atomic-level insights into the interactions between the galloyl moiety and M^{pro}. The galloyl-flavonoids positioned their galloyl groups within the S1' sub-pocket, facilitating hydrogen bonding and π-interactions, particularly with T26 and L27, aligning with experimental studies on previously known inhibitors containing galloyl portions. These findings highlight the galloyl moiety as a key structural feature for enhancing the binding affinity of flavonoids to SARS-CoV-2 M^{pro}, offering valuable insights for the design of potent antiviral agents.



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sciforum-123209: Intrinsically disordered proteins as drug targets

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Protein-Protein Interactions (PPI) mediate numerous processes in cells in health and disease. However, it is extremely challenging to make them drug targets. This is especially true for intrinsically disordered proteins (IDPs). The research in our lab focuses on using peptides for the quantitative biophysical and structural analysis of PPI. Based on this, we develop lead peptides that modulate PPI for therapeutic purposes. In my talk I will give an overview of the research in our lab, including:

Control of condensation and aggregation of proteins by multiphosphorylation: Specific phosphorylation patterns dictate the activity and interactions of many proteins. We developed new methods for rapid and efficient of peptide libraries with distinct multi-phosphorylation patterns. This enables systematic studying at the level of isolated protein domains. We used these tools to elucidate how multi-phosphorylation regulates the self-assembly and activity of two IDPs: the cancer-related APC protein and the neurodegenerative diseases-related Tau protein.

Inhibiting structured and disordered domains simultaneously: We developed a strategy for inhibiting the structured and disordered hot spots of an interaction using chimeric peptides that contain both structured and disordered parts, which target both structured and disordered hot spots at the same protein. The approach is demonstrated for inhibiting the anti-apoptotic iASPP protein.

Specific targeting of cancer cells by a designed peptide: We developed a peptide that specifically targets cancer cells and kills them by activating cell death pathways. The peptide is derived from the NAF-1 protein and acts by selectively penetrating the plasma membrane of cancer cells and targeting their mitochondria-ER network.

Inhibiting protein aggregation in disease: Strategies to inhibit aggregation in disease usually focus on highly specific inhibitors for one hotspot but have not made a clinical impact yet. We developed a systematic approach for designing chaperone-like peptides targeting early aggregation stages populated by dynamic precursors, before a mechanistic differentiation takes place. Peptides designed using this strategy inhibited aggregation of disease-related proteins that aggregate in completely different pathways and may serve as general lead compounds against numerous protein aggregation diseases.



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sciforum-126082: Lasing effect in studies on amyloid oligomers linked with neurodegenerative diseases

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Early detection of neurodegeneration signals is needed before cognitive symptoms appear, to successfully treat neurodegenerative diseases. In neurodegenerative diseases, small, mobile protein aggregates, known as amyloid oligomers, are considered the primary agents of degeneration. Thioflavin T (ThT) is widely used in studying protein aggregation by fluorescence technique, but ThT does not succeed to clearly detect transient states of early-stage protein aggregates. Anyway, since weaker emissions arising from changes of the micro viscosity around protein oligomers have been equally overseen, light amplification through lasing can be used, to enhance their detection sensitivity. Lasing in ThT-stained amyloid oligomers achieves much higher sensitivity than standard fluorescence, allowing for the detection of structural reorganization of the protein involved in disease development. Light amplification has been observed in various states of protein aggregation, and the presence of lasing thresholds enables detection and differentiation of these ones. Furthermore, lasing has been applied to recombinant proteins seeded with human cerebrospinal fluid (CSF), achieving good recognition sensitivity for disease-linked biomolecules. Our studies suggested that lasing should help with early diagnosis of neurodegenerative diseases, potentially before clinical symptoms emerge, which could improve patient outcomes and conditions through timely therapeutic intervention.

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sciforum-123151: Luteolin Mitigates Neuroinflammation of Alzheimer's Disease Through the Activation of Mitophagy

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid- β (A β) plaque deposition and neurofibrillary tangles. While recent FDA approval of amyloid-targeted monoclonal antibodies have shown promise, their efficacy in early-stage AD remains limited. Emerging evidence highlights mitochondrial dysfunction and neuroinflammation as critical drivers of disease progression. Mitophagy, the selective degradation of damaged mitochondria, has emerged as a promising therapeutic target. Additionally, microglia exhibit dynamic polarization states, including disease-associated microglia (DAM), which play a nuanced role in AD pathogenesis. This study investigates the interplay between mitophagy impairment and microglial polarization in AD and evaluates the therapeutic potential of luteolin, a novel mitophagy enhancer identified via high-throughput screening.

Method

We employed Mito-QC reporter mice crossed with 5xFAD mice to generate a transgenic model (Mito-QC-5xFAD) to study mitophagy dynamics in AD pathogenesis. Microglial responses were assessed using BV2 cells or primary microglia. The effects of luteolin and urolithin A on mitochondrial quality control, neuroinflammation, and microglial polarization were systematically evaluated.

Results

Mitophagy impairment was detected in 2-month-old Mito-QC-5xFAD mice, preceding A β plaque accumulation. Treatment with luteolin attenuated mtDNA-STING-dependent neuroinflammation and promoted DAM polarization, enhancing microglial phagocytosis of A β . In vivo studies demonstrated that luteolin reduced STING-mediated neuroinflammation and facilitated A β plaque clearance by modulating microglial polarization in 5xFAD model. These findings position luteolin as a promising therapeutic candidate for targeting mitophagy-microglia crosstalk in AD.

Conclusion

Mitophagy dysfunction is an early hallmark of AD, and its pharmacological activation with luteolin mitigates neuroinflammation and promotes phagocytic DAM polarization. Our study highlights the potential of luteolin as a novel therapeutic agent and underscores the importance of targeting mitophagy-microglia interactions in AD drug development.

Acknowledgement

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sciforum-127464: Machine Learning Driven Techniques for the Discovery of Nrf2/ARE Pathway Modulators for Combating Parkinson's Disease

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder which affects 6.1 million people worldwide. The neuropathological hallmarks include the loss of dopaminergic neurons in the substantia nigra, the presence of Lewy bodies and Lewy neurites caused by α -synuclein aggregation, and neuroinflammation in the brain. Transcription factor nuclear factor E2-related factor 2 (Nrf2, NFE2L2) is an increasingly recognized target for counteracting mitochondrial dysfunction and its consequences in PD. Nrf2 activates the antioxidant response element (ARE) pathway, which includes a range of cytoprotective genes, such as antioxidants and anti-inflammatory genes, and several transcription factors involved in mitochondrial biogenesis.

Methods

We developed a cheminformatics workflow using two ML-QSAR models with PubChem fingerprints and 1D/2D descriptors to predict Nrf2/ARE pathway modulators. Analyzing 606 compounds, we identified key structural features and virtually screened 44,035 compounds for anti-PD activity. Top candidates were validated via molecular docking and dynamics, offering insights into their molecular interactions.

Results

Our random forest-based ML-QSAR models showed high predictive accuracy ($R^2 = 0.9848$ for PubChem fingerprints and 0.986 for 1D/2D descriptors), confirming their robustness for Nrf2/ARE modulators. Key molecular features were identified via VIP and correlation analyses. Molecular docking and dynamics revealed atomic-level ligand-receptor interactions, aligning well with model predictions and offering comprehensive insights into molecular recognition mechanisms.

Conclusion

Our ML-QSAR models align with experimental data; these predictive models establish a powerful computational platform for identifying critical pharmacophoric features, offering a validated framework to identify key pharmacophores and guide rational design of Nrf2/ARE-targeted therapeutics for neurodegenerative diseases.



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sciforum-122718: Multitarget Drug Design for Complex Diseases: Focus on Carbonic Anhydrase Modulators

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Introduction

Combination drug therapies have long been clinically established as effective treatments for multifactorial diseases such as cancer and neurodegenerative disorders, owing to their ability to concurrently engage multiple biological targets. However, recent advances in medicinal chemistry have shifted the focus toward multi-target directed ligands (MTDLs). These single-molecule agents offer significant advantages over traditional combination therapies, including improved pharmacokinetic properties, reduced risk of drug–drug interactions, and enhanced synergistic effects.

Methods

Over the past decade, we have employed advanced structure-based drug design approaches to develop MTDLs incorporating modulators of carbonic anhydrase (CA) –validated targets across various pathological conditions. The compounds were synthesized and evaluated *in vitro* for their ability to modulate CA activity alongside other disease-relevant pathways. Promising candidates demonstrating dual or multitarget efficacy were further investigated in relevant cellular and/or animal models to assess their therapeutic potential.

Results

The resulting MTDLs consistently outperformed corresponding single-target agents and their combinations across multiple disease models. These included glaucoma, cancer, inflammation, infections, and neurodegenerative diseases such as Alzheimer's and other memory disorders. The multitargeting approach led to a more robust and comprehensive therapeutic profile, with enhanced efficacy and target engagement.

Conclusions

Carbonic anhydrase modulators have proven to be highly effective scaffolds in the rational design of MTDLs, supporting their use in addressing the complex pathophysiology of multifactorial diseases. This strategy aligns with current trends in drug discovery, emphasizing integrated therapeutic solutions for conditions such as cancer, infections, and neurodegeneration.



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sciforum-123166: Natural Product-Based Discovery of Antibacterial Agents from *Sophora tomentosa* L. to Tackle Drug Resistance

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Introduction

The rapid rise of antimicrobial resistance (AMR) poses a major global health threat, undermining the efficacy of existing antibiotics. Natural products serve as a valuable source of structurally diverse bioactive compounds with potential for drug discovery. This study investigates *Sophora tomentosa* L., a leguminous plant traditionally used in herbal medicine, as a potential source of antibacterial agents effective against resistant pathogens.

Methods

Methanolic extracts were prepared from the leaves, seeds, and dechlorophyllized leaves of *S. tomentosa*. Bioactivity-guided fractionation using liquid-liquid extraction and column chromatography yielded dichloromethane and ethyl acetate fractions. These were evaluated for antioxidant, antibacterial, and anti-inflammatory activities. Antibacterial efficacy was tested against *Ko-curia rhizophila* and *Bacillus cereus*, while anti-inflammatory activity was assessed via nitric oxide inhibition in RAW 264.7 macrophage cells. GC-MS analysis was performed to identify bioactive compounds.

Results

The dichloromethane and ethyl acetate fractions from leaf and seed extracts (LDF, LEF, SDF, and SEF) exhibited strong biological activities. These fractions significantly inhibited the growth of *K. rhizophila* and *B. cereus*, both associated with foodborne illness and resistance to conventional treatments. Additionally, the active fractions displayed antioxidant properties and inhibited macrophage nitric oxide production, indicating anti-inflammatory potential. GC-MS profiling revealed key phytochemicals, including alkaloids (e.g., matrine), flavonoids (e.g., catechin), phthalic acid derivatives, and fatty acids, many of which are known to disrupt microbial metabolism and resistance mechanisms.

Conclusions

These findings highlight *Sophora tomentosa* as a promising natural source of antibacterial agents with potential applications in overcoming drug resistance. The bioactive compounds identified support its further development as a lead candidate for novel antimicrobial drug discovery.



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sciforum-123171: Simulated microgravity induced changes to drug response, increasing heat shock proteins expression but not apoptosis markers in megakaryoblastic cell line

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Microgravity research is an important part of space biology and human physiology, and has recently been used as a unique platform for drug discovery and cell therapy. Simulated microgravity can alter the chemo-responsiveness of cancer cells, potentially affecting cancer metastasis. Heat shock proteins (HSPs) can be used as biomarkers to assess cellular stress in cells and tissues exposed to microgravity, but our understanding of their role is still incomplete. To investigate the effects of microgravity on cells, we used a desktop random positioning machine (RPM) to simulate microgravity conditions. Our previous studies have shown that simulated microgravity inhibits cell proliferation, induces apoptosis, and changes the morphology and expression of MEG-O1 cell surface markers. According to our data, the main changes in the expression of key apoptosis proteins in megakaryoblastic cells under simulated microgravity conditions occurred after 96 hours. The expressions of BAX and cytochrome C proteins decreased under the influence of doxorubicin, with a more significant decrease in microgravity and with longer exposure times. The expression of BAK decreased mainly after 96 hours under RPM conditions. Non-cleaved caspase 3 and BCL-XL showed no significant changes. However, we found that microgravity-induced drug treatment with doxorubicin led to increased HSP60 expression compared to normal gravity. We also found that cell viability was reduced in microgravity even after treatment with doxorubicin. In simulated microgravity conditions, HSP60 mRNA levels were significantly increased in RPM compared to control cells. These results suggest that microgravity acts as a stressor on cells, leading to increased HSP60 expression both at the mRNA and protein levels. Our results are consistent with the study of apoptosis protein expression in different cells under simulated microgravity conditions. The microgravity-dependent modulation of cancer cells' response to chemotherapy has the potential to impact both space and terrestrial medicine. Biotechnology applications, facilitated by the use of cell and tissue cultures, will help us understand the adverse effects of gravity on health and aid in the preparation of space missions.



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sciforum-123342: Targeting p53-MDM2/MDMX Interaction: Advances in Nutlin Development and Therapeutic Applications

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The tumor suppressor protein p53 plays a central role in apoptosis induction, and its dysregulation is a hallmark of cancer. Under normal conditions, p53 activity is tightly controlled by its primary endogenous regulators, MDM2 and MDMX (MDM4). However, in many cancers, this regulatory balance is disrupted—either through mutations in the TP53 gene or through overexpression of MDM2/MDMX, leading to evasion of apoptosis and uncontrolled tumor growth. Targeting the p53-MDM2/MDMX interaction has emerged as a promising therapeutic strategy, with cis-imidazoline-based compounds, known as nutlins, representing one of the most extensively studied classes of small-molecule MDM2 inhibitors.

Since their initial discovery, nutlins have undergone significant optimization in medicinal chemistry, evolving from early lead compounds to clinical candidates such as RG7112. This work provides a comprehensive analysis of the development of cis-imidazoline-based p53-MDM2/MDMX inhibitors, covering key advancements in their structural design to enhance binding affinity, solubility, and pharmacokinetic properties. Additionally, we explore innovative modifications, including covalent inhibitors, dual-targeting compounds, PROTAC-based degraders, and fluorescently labeled derivatives.

From a synthetic perspective, we evaluated various synthetic routes to nutlin analogs, highlighting scalable methodologies suitable for preclinical and clinical development. Furthermore, we discuss biophysical and biochemical studies that elucidate the binding interactions between nutlins and MDM2/MDMX, including fragment-based approaches for inhibitor design.

Beyond their primary mechanism of action, our comprehensive analysis evaluates extensive research demonstrating the anticancer efficacy of nutlins across diverse tumor cell lines. We also explore emerging evidence suggesting off-target effects and alternative applications of nutlin-derived compounds outside oncology.

By illustrating the evolution of nutlins—from initial hit identification to clinical evaluation—this work serves as an instructive case study in rational drug design and translational research, appealing to both specialists in p53 biology and medicinal chemists engaged in anticancer drug development.



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sciforum-122399: The AR-12 derivative P12-8 enhances autophagy in human microvascular endothelial cells against group A *Streptococcus* infection

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Introduction

Group A *Streptococcus* (GAS) results in invasive diseases, such as necrotizing fasciitis and streptococcal toxic shock syndrome. Despite the administration of antibiotics, mortality rates from streptococcal toxic shock syndrome remain high. Our previous studies have demonstrated that the AR-12 pyrrole derivative, P12-8, inhibits GAS replication directly *in vitro*. Moreover, P12-8 does not exhibit toxic effects on human epithelial A549 cells or human microvascular endothelial cells (HMEC-1) at its minimum inhibitory concentration. In addition to its bactericidal activity, P12-8 also enhances the clearance of intracellular bacteria in GAS-infected A549 and HMEC-1 cells. However, we observed that HMEC-1 cells exhibit relatively poor bactericidal activity against GAS compared to A549 cells. Autophagy plays a crucial role in the defense mechanism of non-phagocytic cells for clearing intracellular GAS. However, unlike epithelial cells, GAS-infected endothelial cells fail to suppress intracellular GAS growth due to defects in selective autophagy. In this study, we further investigated whether P12-8 enhanced autophagy in HMEC-1 cells during GAS infection.

Methods

The bactericidal activity of HMEC-1 cells against GAS following treatment with P12-8 was assessed using a colony-forming unit (CFU) assay. The expression of molecules involved in autophagy and autophagosome formation was evaluated using the confocal microscope.

Results

Using the *in vitro* infection assay, we found that P12-8 significantly decreased the bacterial burden in GAS-infected HMEC-1 cells, an effect that was abolished by autophagy inhibitors such as 3-methyladenine and bafilomycin A1. The results of the immunofluorescence assay showed that P12-8 enhanced the formation of autophagosomes, the acidification of autophagosomes, and the fusion of autophagosomes with lysosomes in GAS-infected HMEC-1 cells.

Conclusions

Taken together, we suggest that P12-8 not only inhibited the GAS growth but also enhanced the bactericidal activity of HMEC-1 cells by promoting autophagy. This study indicates that P12-8 may serve as a potential alternative agent for combating GAS infection.



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Session 6. Physiological Functions of Proteins and Organ Interactions

sciforum-116391: Labeled-Line Circuits of Molecularly-Delineated Sympathetic Neurons for Selective Organ Regulation

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The sympathetic nervous system plays a pivotal role in adapting to environmental changes, a process regulated by spinal sympathetic preganglionic neurons (SPNs) that innervate both postganglionic neurons and the adrenal gland. Despite extensive research supporting the concept of selective control within this system, the neural circuit mechanisms underlying output specificity remain largely unresolved. Combining recent single-cell transcriptomic datasets with viral-genetic tools in mice, we identified three molecularly distinct SPN subtypes within the lower thoracic spinal cord. These subtypes exhibit non-overlapping innervation patterns: one projects specifically to the celiac/superior mesenteric ganglia (CG/SMG), another to the adrenal gland, and a third to the lower sympathetic chains and aorticorenal ganglia (ARG). Chemogenetic manipulation of these SPN subtypes demonstrated selective effects on physiological functions, including gastrointestinal motility regulated by CG/SMG, glucose metabolism mediated by the adrenal gland, and body weight modulation linked to sympathetic control of white adipose tissue and kidney function. This molecularly defined parallel labeled-line organization of sympathetic outflows provides a framework for targeted manipulation of organ-specific functions. Our cFos mapping data further suggest that distinct stressors preferentially activate specific sympathetic outflows. The potential of spatial transcriptomic approaches for mapping active SPN subtypes will also be discussed.

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sciforum-126240: Discovery of the channel synapse and its biological functions

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Chemical neurotransmission is a fundamental mechanism of neural communication, traditionally mediated by Ca^{2+} -dependent exocytosis of synaptic vesicles. However, it has long been known that taste receptor cells in taste buds lack synaptic vesicles, yet still transmit gustatory signals to afferent neurons. We discovered a unique synaptic mechanism in these cells—termed the *channel synapse*—in which neurotransmitter (ATP) release is mediated not by vesicular fusion, but through the ion-conducting pore of a voltage-gated channel complex, CALHM1/3. Structurally, the channel synapse is defined by a tripartite architecture comprising a presynaptic mitochondrion or endoplasmic reticulum, a CALHM1/3-enriched presynaptic membrane, and a closely apposed postsynaptic afferent nerve terminal. In the first part of my talk, I will review the discovery, structural organization, and physiological roles of the channel synapse in the mammalian gustatory system.

The second part will highlight our recent findings that extend the concept of channel synapses beyond the tongue. Using a whole-body screen for CALHM1/3 expression in mice combined with single-cell transcriptomics, we identified novel chemosensory epithelial cells in the throat that form channel synapses with vagal sensory neurons. These cells express GPCRs for noxious chemicals, T2Rs, and mediate airway-protective reflexes: swallowing via hypopharyngeal taste buds and coughing via laryngeal tuft cells. These reflexes were abolished in *Calhm3* knockout mice and could be artificially triggered by optogenetic stimulation of these cells. Moreover, exposure to aeroallergens sensitized the cough reflex in a CALHM3-dependent manner. These findings identify these chemosensory epithelial cells with channel synapses as previously unrecognized end organs of airway protective reflexes and as sites of reflex hypersensitivity, underscoring the broader significance of the channel synapse in sensory physiology and host defense.



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sciforum-124898: Gut-derived glucagon-like peptide-1 induces satiation beneficial for obesity prevention

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Introduction

The GLP-1 receptor agonist was introduced as a new anti-obesity drug in Japan last year. This agent suppresses food intake by acting directly on the brain. In contrast, the gut-derived peptide glucagon-like peptide-1 (GLP-1), which served as the model for this drug, is unstable in vivo, and many aspects of its physiological functions and mechanisms of action remain poorly unclear. We previously identified the zero-calorie rare sugar D-allulose (Allu) as a selective releaser of gut-derived GLP-1. Allu suppresses food intake by activating a subset of vagal sensory neurons expressing GLP-1 receptors (Y. Iwasaki, *Nat Commun*, 2018). However, the central mechanisms mediating this gut GLP-1–vagal signaling remain unknown. In this study, we aimed to identify the hypothalamic mechanism underlying Allu-induced satiety.

Methods and Results

Single peroral administration of Allu activated oxytocin (OXT) and arginine vasopressin (AVP) neurons in the paraventricular (PVH) and supraoptic (SON) nuclei of the hypothalamus, in a manner dependent on vagal sensory nerves and GLP-1 receptors. Next, we selectively suppressed neural activity in PVH^{OXT}, PVH^{AVP}, SON^{OXT}, or SON^{AVP} neurons using chemogenetic approaches with adeno-associated viruses in combination with OXT-Cre or AVP-Cre mice. The Allu-induced anorexigenic effect was significantly attenuated by inhibition of PVH^{AVP} neurons, but not the others. Furthermore, intracerebroventricular administration of a V1b receptor antagonist similarly attenuated the anorexigenic effect. On the other hand, central GLP-1 receptor antagonism blocked the anorexigenic effect induced by GLP-1 receptor agonists but not Allu.

Conclusions

The present study reveals a novel pathway through which endogenous GLP-1 suppresses feeding, involving gut GLP-1–vagal afferent signaling to PVH^{AVP} neurons and subsequent interaction with central V1b receptors. Our findings demonstrate that the anorexigenic mechanisms of pharmacological GLP-1 receptor agonists, which frequently induce adverse effects such as nausea and aversion, are fundamentally distinct from those of endogenous GLP-1 secretion, which suppresses food intake without eliciting aversion. These results underscore the physiological and pathophysiological significance of gut-derived GLP-1–mediated feeding suppression, which is likely to attract increasing attention in future research.



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sciforum-123245: Pemafibrate, a selective peroxisome proliferator-activated receptor α modulator, exerts eNOS-independent protective action against the development of steatotic liver disease in mice

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Background

The progression of metabolic dysfunction-associated steatotic liver disease (MASLD) is influenced by endothelial dysfunction. In both basic and clinical studies, pemafibrate, a selective peroxisome proliferator-activated receptor α modulator, has been shown to alleviate MASLD, but it is uncertain if it is also effective in treating endothelial dysfunction.

Materials and Methods

An MASLD animal model was induced in male wild-type (WT) and endothelial nitric oxide synthase (eNOS)-deficient (eNOSKO) mice by feeding them a high-fat/cholesterol/cholate diet, and they were administered either a vehicle or pemafibrate at 0.17 mg/kg/day for 10 weeks. Biochemical, histological and molecular biological studies were carried out.

Results

Pemafibrate treatment did not alter the plasma lipid profiles of WT or eNOSKO mice, but it decreased plasma AST levels in both WT and eNOSKO mice when compared to those treated with vehicle. In both WT and eNOSKO mice, improvements of MASLD were observed in the pemafibrate-treated WT mice and eNOSKO mice, as demonstrated by histopathological analysis. Furthermore, pemafibrate treatment significantly reduced the expression levels of hepatic NADPH oxidase subunit genes, M1 macrophages, inflammatory cytokine-related genes and profibrotic genes in both WT and eNOSKO mice with reduction of hepatic oxidative stress assessed by dihydroethidium staining and 4-hydroxynonenal protein levels.

Conclusion

In conclusion, pemafibrate can exert protective action against the development of steatotic liver disease regardless of eNOS deficiency.



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sciforum-123054: Ryu-Kaku-San, a herbal medicine, enhances ciliary microbeads transport on ciliated human bronchial epithelia via TRPV4 activation: propagation of metachronal wave

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The ciliary transport is an essential ability of the airways to remove inhaled small particles. Ryu-Kaku-San (RKS, a herbal medicine) is widely used in Japan to enhance antitussive expectorant actions. Ciliated-NHBs (normal human bronchial epithelial cells) were obtained by the air liquid interface culture. Beating cilia were observed by a high-speed video-microscopy and their activities were analyzed by an image analysis program. The activities of cilia were assessed by the beating frequency (CBF) and the bend distance (CBD, amplitude), and the metachronal waves propagated to adjacent cells were assessed by the wavelength. RSK enhanced increases in $[Ca^{2+}]_i$ (intracellular Ca^{2+} concentration) via activations of TRPV4 to increase in CBF slightly, and it decreased $[Cl^-]_i$ (intracellular Cl^- concentration) via activation of Cl^- channels to increase CBD by 50%. Moreover, it enhances propagation of the metachronal wave to adjacent c-NHBs, which was inhibited by HO97047 (an inhibitor of TRPV4), a Ca^{2+} -free solution, a low temperature (25°C) and Gap 27 (an inhibitor of connexin). 4aPDD (an agonist of TRPV4) or ionomycin (IM) propagated the metachronal wave. These indicate that the metachronal wave propagates via connexins activated by $[Ca^{2+}]_i$ increase. The further addition of RKS enhanced the propagation, suggesting that RKS stimulates some signals except Ca^{2+} to enhance the propagation. Ciliated-NHBs express TRPV4 and connexin43. RSK enhanced the ciliary microbead transport. RSK increased velocities of the microbead transport on c-NHBs sheets to 139 %, but it increased to 126% or 121% in a Ca^{2+} -free solution or with HO67047. The present study revealed that the propagation of metachronal wave to adjacent c-NHBs was triggered by an $[Ca^{2+}]_i$ increased via TRPV4. The propagation of the metachronal wave of c-NHBs stimulated by RSK enhances the ciliary microbead transport, suggesting the propagation of the metachronal wave enhances the airway mucociliary clearance.



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sciforum-123221: The Role of Leukotriene B4 Type 2 Receptors in the Healing Process of Anticancer Agent-Induced Enteritis

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The leukotriene b4 (LTB4) type 2 receptor (BLT2), expressed in epithelial tissues such as the skin and intestine, is believed to play a role in intestinal protection and epithelial repair. Intestinal mucositis, a side effect of anticancer agents, often persists even after treatment has ended, but the factors involved in the healing process remain unclear. Therefore, the aim of this study was to develop a model of anticancer agent-induced enteritis healing, clarify the role of BLT2 in the repair of intestinal epithelium, and propose a new treatment for enteritis. Male C57BL6 mice were treated with 5-fluorouracil (5-FU, 30 mg/kg) once daily for five consecutive days, followed by LY255283 (a BLT2 antagonist) for four days. 5-FU at 30 mg/kg did not affect body weight and fecal changes but delayed histological recovery of the small intestinal epithelium. Furthermore, we performed survival cell counts, a wound healing assay, and a transwell migration assay with 5-FU, CAY10583 (BLT2 agonist), and LY255283 in a rat small intestinal epithelial cell line (IEC-6). Treating IEC-6 cells with the BLT2 agonist promoted cell proliferation in a concentration-dependent manner. However, BLT2 antagonists did not inhibit cell proliferation unless applied at high concentrations. Additionally, treatment with the BLT2 agonist enhanced cell migration, while BLT2 antagonist treatment inhibited it. This suggests that LY255283 may inhibit cell migration without affecting cell proliferation; LY255283 treatment also inhibits wound healing in IEC-6 cells at concentrations that do not affect cell proliferation. These findings suggest that inhibition of BLT2 delays the healing of 5-FU-induced enteritis, indicating that BLT2 may be a target for the prevention and treatment of anticancer agent-induced enteritis.



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sciforum-126487: Acetylated derivative of nobiletin may alleviate Alzheimer-like cognitive impairment through PI3K/AKT signaling pathway and gut microbiota in mice

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Methylglyoxal, one of the major precursors of advanced glycation end products (AGEs), plays a relevant role in the pathophysiological connection between diabetes and Alzheimer's disease. Our previous studies have shown that acetylation enhanced the distribution of citrus polymethoxyflavone in mouse tissues, as well as in the brain. In the present study, we investigated the neuroprotective effects of 5-acetoxy-6,7,8,3',4'-pentamethoxyflavone (5-AN), an acetylated derivative of nobiletin, against methylglyoxal-induced Alzheimer-like cognitive dysfunction in mice. Chronic methylglyoxal treatment (1% in drinking water for 6 weeks) significantly impaired spatial memory in mice, as shown by increased escape latency, reduced time in the target quadrant, and fewer target crossings in the Morris water maze test ($p < 0.05$). Administration of 5-AN improved methylglyoxal-induced spatial learning impairment and memory decline symptoms. In addition, 5-AN reduced the hyperphosphorylation of tau protein, aggregation of A β plaque and neuronal apoptosis induced by methylglyoxal and activated the PI3K/Akt signaling pathway in the hippocampus of mice. Furthermore, 5-AN also ameliorated the gut microbiota dysbiosis induced by methylglyoxal in mice. In summary, our results suggest that 5-AN may have the potential to improve cognitive impairment and brain nerve damage, and its mechanism may be achieved through activating the PI3K pathway and improving gut flora.



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sciforum-122434: Characterization of the content of endothelial extracellular vesicles derived from group A *Streptococcus*-infected human dermal microvascular endothelial cells

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Introduction

Group A *Streptococcus* (GAS) infection can lead to severe invasive diseases, such as necrotizing fasciitis, which is characterized by high mortality rates. These infections are frequently complicated by bacteremia, thrombosis, and endothelial dysfunction, which consequently hinder antibiotic delivery and reduce treatment efficacy. Endothelial dysfunction induced by bacteremia leads to alterations in the release of endothelial extracellular vesicles (EVs). EVs, containing molecular fingerprints that reflect their sources and the different stages of infection, have significant potential for use in the development of reliable diagnostic markers to monitor infections. In this study, we compared EVs, with a particular focus on their microRNA (miRNA) and von Willebrand factor (vWF) content, both of which are known to be associated with thrombosis formation and endothelial dysfunction, collected from uninfected and GAS-infected human dermal microvascular endothelial cell line-1 (HMEC-1) cells.

Methods

vWF content was analyzed using Western blotting. The size distribution of EVs was analyzed using nanoflow cytometry. Small RNA sequencing was performed to identify miRNAs. Gene Ontology analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis were conducted to assess the functionality and significance of the identified exosomal miRNAs. The miRTarBase database was used to predict the target genes regulated by these miRNAs.

Results

The sizes of EVs collected from uninfected and GAS-infected HMEC-1 cells ranged from 60 to 100 nm. These EVs exhibited exosomal markers, including CD63 and TSG101, and contained vWF and miRNAs. Analysis of the vWF content within EVs indicated no significant difference between the two groups. However, comparison of miRNA content revealed that 101 miRNAs were upregulated, while 167 miRNAs were downregulated after GAS infection. Predictive analysis of the target genes regulated by these miRNAs showed that 904 unique targets regulated by upregulated miRNAs, 3,852 targets regulated by downregulated miRNAs, and 524 shared targets regulated by both.

Conclusions

These findings suggest that GAS infection modified the miRNA composition of endothelial cell-derived EVs, which might influence gene expression in neighboring cells and the progression of the infection.



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sciforum-122329: Des-aspartate Angiotensin 1 (DAA-1) as a Novel AT1R Modulator: In-Silico and Vascular Functional Insights

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Introduction

Des-aspartate angiotensin-1 (DAA-1) is an endogenous peptide with emerging therapeutic potential in cardiovascular diseases. Known for its anti-inflammatory and vasodilatory effects, DAA-1 is hypothesised to interact with angiotensin II type 1 (AT1) receptors. While prior experimental studies suggest receptor involvement, computational evidence is lacking. Therefore, the study aims to (1) examine and validate the binding potential of DAA-1 to AT1 and its various interactions with the amino acids of the receptors. The next (2) is to validate the in silico findings using a functional vascular investigation.

Methods

Molecular docking was performed using AutoDock Vina in Chimera, with interaction visualization via BIOVIA Discovery Studio. Binding sites on AT1 and AT2 receptors were predicted using co-crystallized ligand coordinates. Functional assays were conducted using wire myography on isolated arteries from lean and high-fat diet-induced obese Sprague-Dawley rats.

Results

The in-silico analysis demonstrated a high binding affinity of DAA-1 for the AT1R (-11.2 Kcal/mol), surpassing endogenous angiotensin II (-10.8 Kcal/mol) and losartan (-10.1 Kcal/mol). Molecular dynamics simulations confirmed stable receptor-ligand interactions with favourable RMSD and RMSF profiles. Binding free energy calculations supported the strong thermodynamic feasibility of DAA-1 binding. Vascular functional assays revealed a biphasic effect, with vasodilation at the lower dosage of DAA-1 (1 fM to 1 nM) and vascular contractility was observed at higher dosage (1 nM to 1 μ M).

Conclusion

DAA-1 exhibits a unique vascular action by selectively modulating AT1 receptor signalling, which is expressed in both arteries and perivascular adipose tissue (PVAT). This promotes vasodilation while preserving beneficial receptor functions. These findings suggest its potential as a next-generation therapeutic for obesity-associated hypertension. Further clinical studies are warranted to validate its therapeutic efficacy.



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sciforum-126379: Development of a *Centella Asiatica* - Based Antibacterial Spray to Inhibit the Growth of *Listeria Monocytogenes* on Food

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Foodborne pathogen, *Listeria monocytogenes*, causes listeriosis with a death toll of 20%–30% (Osek et al, 2022). The disease is comparatively rare with 0.1 to 10 cases per 1 million individuals per annum based on the countries and regions of the globe (Zahid et al, 2023), resulting in serious life-threatening conditions like meningitis, and encephalitis in ready-to-eat (RTE) foods, meat, and dairy products. RTE foods are becoming common due to their ease of convenience, long life, and ease of direct consumption without final heat or antimicrobial treatment between production and consumption implies that any contamination due to bacteria can go directly to the consumers. Hence, the possibility of developing food science technology to enhance the wholesomeness of RTE foods – from intelligent packaging to plant-derived preservatives must also be considered. One of the promising ideas to this end is to search for traditional medicinal herbs dating back to the ancient Indian (Ayurveda) and Chinese medicinal systems, *Centella asiatica*. The plant leaves could prove to be a potential source of volatile oils, triterpenoids, and phenolic compounds, particularly flavonoid to impart to it antimicrobial and antioxidant activities.

The study involves the extraction of bioactive compounds like triterpenoids plus saponins from *Centella asiatica* for it uses food grade ethanol. These extracts are formed into a food-safe spray and are tested for antibacterial activity for *Listeria monocytogenes*. Agar diffusion methods along with MIC (Minimum Inhibitory Concentration) analysis are used in the testing. Food quality changes based on formula when sensed by evaluators via taste, aroma, texture through cytotoxicity tests. Samples of soft cheese were used as a test subject. Both treated and untreated cheese samples were stored in the refrigerator and under monitoring for any bacterial growth in a week.

Experimental data indicated that the growth of bacteria on the sprayed soft cheese was slower than the not sprayed sample. Under 7 days of refrigerated storage, the treated cheese had a growth in bacteria of just 1.2 log CFU/g, while the control had 2.8 log CFU/g—representing a reduction in growth of 57.1%.



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sciforum-126434: Dynamic Regulation of Zinc Transporters During Experimental Ileitis in Mice

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Introduction

Crohn's disease (CD) frequently causes severe inflammation in the terminal ileum. Although the 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis model is commonly used to investigate the pathogenesis of CD, structural and functional differences between the colon and ileum have led to a considerable gap between such models and the clinical pathology of CD. Low serum zinc levels are often observed in patients with CD, and TNBS-induced colitis is aggravated in zinc-deficient mice. It is evident that maintaining zinc levels is crucial for suppressing intestinal inflammation, but the specific zinc transporter involved in this process remains unclear. Therefore, we aimed to analyze inflammation-associated changes in the expression of zinc transporters using a TNBS-induced ileitis model in mice.

Methods

Anesthetized male C57BL/6N mice were injected ileal lumenally with 1.5 mg of TNBS in 50% ethanol. At 6, 24, and 48 hours post-injection, mice were sacrificed to analyze the inflammatory response, serum zinc levels, and changes in *Zip* mRNA expression.

Results

The mRNA expressions of *tnf-α* and *il-1β* significantly increased in the ileum tissue in the TNBS-injected groups compared with the non-treated group (control), with a peak at 24 hours after injection. Serum zinc levels were reduced, accompanied by decreased expression of *zip4* and *znt1* in the ileum. The expression of *zip7* also significantly decreased at 24 hours but recovered to control by 48 hours, coinciding with starting ileal re-epithelialization. In contrast, the expressions of *zip4* and *znt1* in the duodenum were significantly increased in response to the reduction of serum zinc levels.

Conclusion

TNBS-induced ileitis peaked 24 hours after the challenge and significantly decreased serum zinc levels, with decreased expressions of *zip4*, *zip7*, and *znt1* in the ileal tissue. Upregulation of *zip4* and *znt1* in the duodenum and/or *zip7* in the ileum may mitigate the pathogenesis of CD and lead to the extension of the remission maintenance period.



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sciforum-123101: Enhancing CFTR facilitates osteoporotic bone fracture healing in mice

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Introduction

Cystic fibrosis transmembrane conductance regulator (CFTR), a Cl⁻ channel gene, is noted to be important for bone health, the molecular mechanisms underlying which remain unclear. Given the reported age-related downregulation of CFTR gene, we tested possible effect of enhancing CFTR in treating osteoporotic bone fractures and explored possible mechanism.

Methods

Femoral bone fracture surgery was done in ovariectomized female mice to mimic age-related osteoporosis and bone fracture. CFTR enhancers or vehicle controls were locally injected into the fracture site in the mice every two days for 2–4 weeks. In the recovery period, fracture callus size was monitored in the mice by X-ray. Micro-CT and four-point bending tests were performed at 8–12 weeks post-fracture to evaluate the effect of CFTR enhancers on fracture repair. In addition, MLO-Y4, a mouse osteocyte line, was used, where CFTR was knocked out by CRISPR-Cas9. Cl⁻ and Ca²⁺ imaging as well as transcriptome analysis were conducted in the cells.

Results

X-ray results revealed that the largest callus was formed at 2 weeks post-fracture in all groups. Callus morphometry showed that, at 2 to 8 weeks post-fracture, mice treated with CFTR enhancers had larger callus areas and greater callus widths, suggesting more active repair compared to the control group. The CFTR enhancers-treated group also exhibited better fracture alignment (indicated by micro-CT) and stronger mechanical strength, compared to the control group. MLOY4 cells lack of CFTR exhibited defective Cl⁻ transport and impaired Ca²⁺ mobilization, as well as proinflammatory transcriptomic changes.

Conclusion

Together, these results have suggested the potential of enhancing CFTR in facilitating osteoporotic bone fracture healing. Suppressing inflammation might underlie the protective role of CFTR in skeletal homeostasis. (The work is supported in part by Health and Medical Research Fund of Hong Kong (No.18191361) and Areas of Excellence (No. AoE/M-402/20)).



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sciforum-125812: FUT8, a glycosyltransferase, mediates cardiac dysfunction during isoproterenol-induced heart failure mouse models

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Cardiovascular diseases, such as Hypertension or myocardial infarction, induces myocardial cell death and excessive fibrosis, resulting in heart failure. Therefore, developing regulatory methods to control not only cardiomyocyte death but also cardiac fibrosis remains a crucial and unresolved issue. Recently, it has been reported that expression of Fucosyltransferase 8 (FUT8), a glycosyltransferase, is increased in the hearts of mouse models of heart failure. However, the specific role of FUT8 in cardiac fibrosis and cardiac dysfunction remains unclear. In this study, we investigated the role of FUT8 in the heart using FUT8 overexpressing mice (FUT8 Tg). Mouse models of heart failure were created by chronic subcutaneous administration of isoproterenol (ISO), a nonselective β -adrenergic agonist, via osmotic mini-pump. Echocardiogram analysis revealed that overexpression of FUT8 alone (FUT8 Tg-PBS) reduced contractile function compared to WT-PBS (control), while treatment of FUT8 Tg mice with ISO (FUT8 Tg-ISO) resulted in further dysfunction, comparable to that observed in WT-ISO. Heart weight/Body weight ratio was no different between FUT8 Tg-PBS and WT-PBS, whereas it was lower in FUT8 Tg-ISO compared to WT-ISO. Fibrotic areas increased in FUT8 Tg-PBS compared to WT-PBS, and tended to increase in FUT8 Tg-ISO more than in WT-ISO. Gene expression of Collagen Type I alpha 1 chain (*Col1a1*), a marker of fibrosis, and alpha Smooth Muscle Actin (*α -SMA*), a marker of fibroblast to myofibroblast differentiation, were analyzed by quantitative RT-PCR. FUT8 Tg-PBS induced increment of *Col1a1* expression compared to WT-PBS, while *α -SMA* expression remained unchanged. Additionally, *Col1a1* and *α -SMA* expression were similarly increased in FUT8 Tg-ISO compared to WT-ISO. These results suggest that FUT8 is involved in cardiac collagen production, leading to decrease in contraction but does not affect fibroblast differentiation. Further studies, including histological and comprehensive molecular analyses and effects on cardiomyocytes, will be conducted to better understand the role of FUT8 in heart failure.



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sciforum-124879: Gut hormone cholecystokinin drives oxytocin-dependent thermogenesis in brown adipose tissue via vagal sensory nerve–brain axis

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Introduction

Thermoregulation is a fundamental autonomic function essential for the survival of homeothermic animals. Although vagal sensory nerves are well known to relay visceral signals in feeding behavior, metabolic, gastrointestinal, and immune regulation, their role in body temperature control remains poorly understood. The gut hormone cholecystokinin (CCK) is a potent activator of vagal sensory nerves. It is released postprandially and acts via CCK_A receptors (CCK_AR) expressed on these nerves, thereby conveying satiation signals to the brain. In addition to its anorexigenic effects, CCK reportedly enhances brown adipose tissue (BAT) thermogenesis. However, the involvement of vagal sensory nerves and central mechanisms in this effect remains unclear. In the present study, we investigated the thermogenic effects of intraperitoneally (IP) administered CCK and examined the involvement of vagal sensory nerves, hypothalamic neurons, sympathetic nerves, and BAT in this mechanism.

Methods and Results

We examined whether peripheral administration of CCK increases rectal temperature in mice and investigated the roles of vagal sensory nerves and sympathetic nerves innervating interscapular BAT in this thermoregulatory response. CCK-8 transiently and dose-dependently elevated rectal temperature. This thermogenic effect was abolished by pretreatment with CCK_AR antagonist, sub-diaphragmatic vagotomy, or conditional knockout of CCK_AR in vagal sensory neurons using Phox2b-Cre; Cckar-flox/flox mice. Electrophysiological recordings showed that CCK-8 increased sympathetic nerve activity to interscapular BAT. Furthermore, CCK-8-induced thermogenesis was completely blunted by BAT-targeted sympathectomy or β 3-adrenergic receptor blockade, as the β 3 receptor is essential for BAT thermogenesis. To identify the central relay, we performed fiber photometry and observed that CCK-8 transiently activated oxytocin neurons in the hypothalamic paraventricular nucleus (PVH^{OX}). Chemogenetic inhibition of PVH^{OX} neurons via AAV-mediated hM4Di expression markedly attenuated CCK-induced thermogenesis. Finally, intracerebroventricular injection of an oxytocin receptor (OXTR) antagonist suppressed the thermogenic response.

Conclusions

The present study demonstrates that exogenous CCK increases core body temperature through a vagal sensory nerve–brain–BAT sympathetic pathway, with PVH^{OX} neurons and central OXTR signaling playing critical roles in this thermogenic mechanism. These findings may also provide insight into a physiological mechanism by which nutrient sensing in the gut induces endogenous CCK secretion and drives diet-induced thermogenesis.



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sciforum-122798: Histone chaperone CHAF1B facilitates germinal center B cell differentiation via cooperation with BCL6 and TBL1XR1

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BCL6 is a master regulator of germinal center (GC) B cells. Further identification and characterization of factors that may play a role within the BCL6 network is important for our understanding of GC B cell differentiation and function. Germinal centers are dynamic structures induced by T cell-dependent antigen stimulation during the humoral immune response. They exhibit a unique phenotype characterized by extensive proliferation and tolerance of genomic damage, which occurs as a byproduct of somatic hypermutation to enable immunoglobulin affinity maturation. All these events share dependencies on the same transcriptional regulatory network of BCL6, which plays a suppressive regulatory role by recruiting corepressor proteins. Our study identified CHAF1B as a novel partner of the BCL6 complex in GC B cells, promoting GC formation and humoral immunity. The loss of CHAF1B impairs dark zone and light zone organization, and leads to abnormal GC responses and antibody production. Mechanistically, CHAF1B stabilizes the BCL6/TBL1XR1 complex, facilitating GC B cell differentiation through cooperative transcriptional repression. Moreover, CHAF1B overexpression may reduce plasma cell generation by prolonging the GC response, which is positively correlated with the production of high-affinity antibodies induced by vaccines or pathogens. These findings not only advance the understanding of GC biology but also had potential implications for developing targeted strategies to improve vaccination efficacy.



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sciforum-126174: Illuminating Vitamin D's Regulatory Influence on Chloride Ion Dynamics in Zebrafish

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This investigation reveals a significant, previously under-appreciated function for vitamin D in managing chloride ion levels within the freshwater zebrafish model, extending its established roles in calcium metabolism and acid-base balance. Freshwater aquatic species continuously counteract passive chloride loss by actively acquiring this essential anion from their dilute surroundings. Our findings demonstrate that exposure to environments with diminished chloride concentrations triggers an elevation in the circulating levels of the active vitamin D metabolite, $1\alpha,25(\text{OH})_2\text{D}_3$, in these fish. Subsequent experiments involving the direct administration of $1\alpha,25(\text{OH})_2\text{D}_3$ led to a discernible increase in the total body chloride content of zebrafish. Mechanistically, this study links vitamin D activity to the enhanced genetic and protein expression of key chloride transport proteins, specifically the Na^+-Cl^- cotransporter 2b (NCC2b) and the chloride channel 2c (CLC2c). Furthermore, vitamin D treatment resulted in a proliferation of the specialized ionocytes responsible for expressing these transporters. The critical involvement of vitamin D signaling pathways was confirmed by identifying its receptors (VDRs) within NCC2b-positive ionocytes and by observing that targeted disruption of VDR function abrogated the vitamin D-mediated upregulation of NCC2b and CLC2c, along with the associated increase in ionocyte populations. These results collectively establish vitamin D as a pivotal hormonal factor influencing chloride uptake mechanisms in zebrafish, offering new perspectives on its multifaceted physiological importance in fish.



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sciforum-122185: Isoflavones regulate reactive oxygen species-induced angiotensinogen release in proximal tubule cells

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Introduction

The renin-angiotensin system (RAS) is a regulatory mechanism of the endocrine system involved in controlling blood pressure, body fluid volume, and body electrolytes. We previously showed that the tissue RAS in the kidney is important in the onset and progression of kidney disease. In particular, we focused on the angiotensin precursor angiotensinogen (AGT), located upstream in the renin-angiotensin pathway, and reported that urinary angiotensinogen is a useful early marker of diabetic nephropathy. However, the mechanism by which renal angiotensinogen expression and increased secretion are controlled in the onset of diabetic nephropathy remained unclear. In this study, we investigated factors that regulate angiotensinogen secretion using renal proximal tubule cells, where angiotensinogen expression has been confirmed. We hypothesized that dietary components with high antioxidant capacity affect renal angiotensinogen expression, and aimed to determine whether antioxidants such as polyphenol isoflavones suppress the angiotensinogen-mediated mechanism of diabetic nephropathy in proximal tubule cells.

Methods

The isoflavone daidzein and its metabolite equol were added to cultured human proximal tubule cells prior to their stimulation with reactive oxygen species (ROS). After 24 hours, the angiotensinogen protein concentration of the culture medium was determined by enzyme-linked immunosorbent assay, and angiotensinogen mRNA levels of the cells were measured by real-time PCR. The intracellular signal transduction mechanism was investigated by the inhibitors of mitogen-activated protein kinase (MAPK) cascade. Additionally, an attempt was made to search downstream of AGT.

Results

The stimulation of human proximal tubule cells with hydrogen peroxide led to an increase in angiotensinogen mRNA expression and protein secretion, which were positively correlated with the hydrogen peroxide concentration. The addition of polyphenol isoflavones prior to hydrogen peroxide stimulation significantly decreased angiotensinogen mRNA expression and protein secretion. MAPK cascade protein analysis suggested that p38 may be involved in the underlying intracellular signal transduction.

Conclusions

ROS stimulation increased angiotensinogen secretion by proximal tubule cells, and this increase was attenuated by the antioxidant isoflavones daidzein and equol.



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sciforum-125896: Monitoring Heatstroke: A Proteomics-Based Multibiomarker Approach to Assess Potential Post-Heatstroke Complications

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Global warming increases heatstroke frequency and severity, posing a global health threat. Heatstroke causes inflammatory and coagulation symptoms, potentially leading to central nervous system lesions and multi-organ damage. Notably, around one-third of heatstroke cases develop acute kidney injury (AKI), which contributes to chronic renal impairment and increased mortality. Despite this, current treatments mainly target acute symptoms, with limited focus on long-term recovery. This study is designed with a bidirectional, time-point approach to investigate heatstroke recovery using clinical samples and rat models to identify early biomarkers and monitor complications. Plasma samples were collected from patients at diagnosis and recovery, while SD rats were exposed to graded heat stress (control, mild [core body temperature = 41.5°C], and severe [core body temperature = 42.3°C]) to model disease progression.

Proteomic profiling involved 2-DE-based protein separation, LC-MS/MS identification, Western Blot validation, and network analysis. In human samples, five proteins showed significant longitudinal changes: downregulation of alpha-1-antitrypsin (A1AT) and alpha-1-microglobulin/bikunin precursor (AMBP/A1M), and upregulation of apolipoprotein A-IV (APOA4), complement component 2 (C2), and clusterin. Notably, AMBP/A1M reduction was confirmed in both monomeric (A1M) and complexed (A1M-IgA) forms. In rats, 14 proteins were differentially expressed across severity groups, with functional enrichment in actin cytoskeleton regulation, amino acid metabolism, immune response, and energy metabolism. Key proteins included alpha-1-macroglobulin, albumin, phosphoglycerate mutase 1 (Pgam1), gelsolin, T-kininogen 1, aspartate aminotransferase (Got1), and complement component 3 (C3), including its fragments C3a and C3b. This study highlights key pathways involved in heatstroke pathophysiology, including complement and coagulation cascades activation, lipid metabolism, cytoskeletal remodeling, and metabolic regulation on energy and amino acid biosynthesis. By integrating results from clinical and experimental models, this study proposes a panel of potential plasma biomarkers to assess post-heatstroke severity, enabling early intervention strategies and improved long-term outcomes.



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sciforum-123020: Ovarian granulosa-specific knock-out of multidrug resistance protein 4 (MRP4) induces female subfertility by disturbing late folliculogenesis and estrus cycle in mice

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Introduction

Ovarian granulosa cells are essential to folliculogenesis and steroidogenesis, the molecular control of which, however, has not been fully understood. Previously, we demonstrated in the endometrium/uterus that multidrug resistance protein 4 (MRP4), also known as ATP-binding cassette family C member 4 (ABCC4), plays an important role in regulating Wnt/ β -catenin and prostaglandin E₂ (PGE₂) signaling pathways. Given these pathways also important to ovarian physiology, we hypothesized that MRP4 might be expressed in ovarian granulosa cells to play a role in ovarian functions.

Methods

In the present study, we utilized two granulosa cell-specific Cre mouse transgenic lines, *Cyp19a1^{cre}* and *Amh^{cre}*, to cross with another transgenic mouse line where the exon of MRP4 gene (*Abcc4*) is flanked by LoxP sites (*Abcc4^{fl/fl}*), and successfully created two mouse models of granulosa cell-specific knockout of MRP4 (*Abcc4^{fl/fl}Cyp19a1^{cre}* and *Abcc4^{fl/fl}Amh^{cre}*). Follicles at different developmental stages of folliculogenesis, namely, primary, secondary, preantral, early antral and late antral follicles were identified and counted through morphological assessment. Daily vaginal smear and cytology were performed in the mice to identify stages of estrus cycle, namely, proestrus, estrus, metestrus and diestrus. Fertility tests of the mice were conducted by mating with wild-type male mice for over 12 months. Additionally, primarily cultured mouse granulosa cells were used.

Results

Results showed that, as compared to the control, both *Abcc4^{fl/fl}Cyp19a1^{cre}* and *Abcc4^{fl/fl}Amh^{cre}* mice showed a decreased number of late antral follicles, disrupted estrus cycle with significantly shortened estrus phase. Fertility tests revealed significant reduction in pups' number in *Abcc4^{fl/fl}Amh^{cre}* mice compared to the controls. In primarily cultured mouse granulosa cells, siRNA-based knock-down of MRP4 caused defective PGE₂ secretion and impaired estrogen production, as well as proteomic changes.

Conclusion

Taken together, the present results have suggested a previously undefined role of MRP4 in regulating granulosa cells for folliculogenesis and steroid homeostasis, providing new insights into the understanding of granulosa cell functions as well as related ovarian disorders. (*The work was supported by General Research Fund (No.15102622) from The Research Grant Council of Hong Kong.*)



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sciforum-126345: Prediction of Ischemic Stroke Using Serum Antibody Markers with a Machine Learning Model

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Introduction

We previously identified antibody markers from serum samples collected within two weeks of ischemic stroke onset. The antibody markers target antigens which the body had been exposed prior to stroke. Several of these markers have demonstrated individually useful for diagnostic prediction. In this study, we aimed to enhance diagnostic accuracy and verify the utility of antibody markers by applying machine learning models to a large-scale cohort, incorporating multiple antibody markers and clinical information.

Methods

A total of 835 participants (285 healthy controls and 549 patients with acute ischemic stroke) were analyzed. We measured antibody levels against three markers previously validated for their association with stroke pathogenesis through protein function analysis. Antibody levels were obtained using an AlphaLISA-based immunoassay and analyzed with background clinical data (age, sex, medical history). We conducted the analysis using Python by implementing machine learning models, and evaluated model performance. A random forest model was applied, and classification accuracy was quantitatively assessed using the area under the receiver operating characteristic curve (AUC). Additionally, feature importance analysis was performed to examine the contribution of antibody markers to classification.

Results

The random forest model achieved a high diagnostic accuracy with an AUC of 0.87. A hyperparameter-optimized model using grid search also yielded an AUC of 0.87. By feature importance, antibody markers were the second most influential predictors after age.

Conclusion

Machine learning models enabled highly accurate prediction of ischemic stroke onset in a large-scale cohort. Antibody markers were validated as key predictors in diagnostic modeling.



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sciforum-122215: Probiotic *Lactobacillus rhamnosus* GG Attenuates PM2.5-induced Respiratory Inflammation and Modulates Cytokine Responses

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Respiratory allergies and inflammatory diseases have become increasingly prevalent worldwide, significantly impacting public health and quality of life. Among environmental factors, air pollutants, particularly fine particulate matter (PM2.5), have emerged as critical contributors to exacerbating acute and chronic respiratory inflammation. Complete elimination of environmental allergens and pollutants remains challenging, emphasizing the urgent need for alternative therapeutic strategies to alleviate symptoms, minimize dependence on conventional pharmacological treatments, and reduce potential adverse drug reactions and resistance issues. Probiotics, specifically *Lactobacillus rhamnosus* GG (LGG), have recently attracted considerable attention due to their immunomodulatory properties. Despite promising preliminary evidence, the efficacy and underlying mechanisms by which LGG may alleviate PM2.5-induced respiratory inflammation have not been thoroughly elucidated. This study aimed to investigate the therapeutic potential of LGG supplementation in mitigating airway hyperresponsiveness (AHR) and inflammatory responses triggered by PM2.5 exposure using a well-established animal model. Employing comprehensive assessments of immune cell infiltration, inflammatory markers, histological changes, and cytokine profiling, our findings revealed that LGG administration markedly reduced airway hyperresponsiveness and significantly alleviated respiratory inflammation compared to untreated controls. Histological examination demonstrated a substantial reduction in inflammatory cell infiltration, particularly eosinophils and neutrophils, within lung tissue samples of LGG-treated subjects. Additionally, cytokine profiling highlighted notable downregulation of critical pro-inflammatory cytokines, including IL-4, IL-5, IL-13, IL-8, IL-17, and IL-25, suggesting broad-spectrum immunomodulatory effects. Collectively, these results demonstrate that LGG supplementation effectively mitigates PM2.5-induced respiratory inflammation, improves airway responsiveness, and holds substantial promise as a preventive and adjunctive therapeutic intervention for respiratory inflammatory diseases exacerbated by environmental pollutants.



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sciforum-123232: Profiling the Genetic Interaction Landscape in Bacteria

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Gene redundancy often complicates systematic approaches to characterizing gene functions because single gene deletions may not produce discernible phenotypes. Thus, despite the advent of next-generation sequencing, over 11 million microbial genes in the NCBI reference sequence database have no known function. In this study, we report Dual Tn-seq, a novel platform for comprehensively assaying the fitness of a large pool of double mutants in parallel. Dual Tn-seq couples random barcode transposon-site sequencing (RB Tn-seq) with the Cre-lox system, enabling deep sampling of ~1.4 billion double mutants in the human pathogen *Streptococcus pneumoniae*. This work captured 73% of the 1.3 million combinations of gene deletions that could theoretically be made in a single genome. The genetic interactions identified spanned a wide range of biochemical processes, revealing new factors in presumably well-studied pathways. As a proof-of-concept, we discovered a new class of CTP synthase, a regulator controlling peptidoglycan synthesis, factors involved in nucleotide transport and amino acid synthesis, etc. Furthermore, we revealed the potential cellular roles of 69 uncharacterized proteins by identifying their genetic interactions with and genes involved in known biological processes. In addition, the double-mutant library can be easily adapted to other growth conditions to investigate an even broader range of genetic interactions. Since Dual Tn-seq does not require the construction of a large array of single mutants, it can easily adapt to various microorganisms.



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sciforum-122686: Rab27-dependent release of extracellular vesicles facilitates the secretion of the dengue virus non-structural protein 1

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Dengue fever is a significant arthropod-borne disease worldwide, resulting in approximately 20,000 yearly deaths. The non-structural protein 1 (NS1) of the dengue virus (DENV) is the major non-structural protein secreted by infected cells and is highly correlated to the pathogenesis of severe dengue cases. Multiple studies have indicated that the secreted NS1 increases vascular permeability, causing coagulopathy, activating immune cells, and triggering inflammation. These findings suggest that secreted NS1 plays a pathological role, although the exact secretory pathway remains unclear. Increasing evidence indicates that virus-associated extracellular vesicles (EVs) contain not only viral components, such as viral proteins, genetic material, and virion particles but also components from the host. Rab27 is one of several GTPases and is essential to regulating EV release by affecting the docking of the multivesicular body (MVB) to the target plasma membrane. We demonstrate that NS1 can be exported from DENV-infected cells by EVs. Additionally, we assess the topology of NS1 and reveal that it is associated with the surface of EVs. NS1 and EV biogenesis-associated proteins ALG-2-interacting protein X (ALIX) and neutral sphingomyelinase2 (nSMase2) co-accumulate in extracellular vesicles. In addition, the secretion of DENV NS1 is regulated by Rab27. The treatment with the EV biogenesis and secretion inhibitor, Tipifarnib, reduces NS1 and nSMase 2 levels in the EVs but does not affect ALIX. This study provides new insights into the mechanisms of DENV NS1 secretion through Rab27-mediated vesicle trafficking and valuable therapeutic targets for dengue disease.



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sciforum-122186: Regulation of angiotensinogen secretion by reactive oxygen species in mesangial cells

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Introduction

The renin-angiotensin system (RAS) regulates blood pressure and body fluid and electrolyte status. We have shown that the RAS in the kidney is important for the onset and progression of renal disease. However, the mechanisms whereby the expression and secretion of AGT are dysregulated during the onset of diabetic nephropathy remain unclear. We also hypothesized that highly antioxidant components in foods affect the expression of angiotensinogen in the kidney, and aimed to clarify, using mesangial cells, whether antioxidants suppress the mechanism of diabetic nephropathy mediated by angiotensinogen. This study was also conducted in proximal tubule cells, which strongly express AGT, but we examined mesangial cells, where AGT expression is also observed.

Methods

Human mesangial cells were stimulated by active oxygen. Daidzein, an isoflavone, and its metabolite equol, were added 30 minutes before the stimulus. After 24 hours, the culture medium was collected and the AGT concentration was measured by ELISA. RNA was extracted from the cells, and AGT expression was measured using real-time PCR. The antioxidant capacity of daidzein and equol was assessed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Additionally, an attempt was made to search downstream of AGT.

Results

Daidzein and equol showed antioxidant activity, with DPPH radical scavenging abilities of 59.3% and 61.2%, respectively. The secretion of AGT by mesangial cells was increased with increasing hydrogen peroxide concentration. Stimulation with 10 mM hydrogen peroxide increased AGT secretion and also increased the activity of catalase, which removes reactive oxygen species. However, when the isoflavones daidzein and equol, which are polyphenols with antioxidant properties, were added to the mesangial cells at the same time, the secretion of AGT and the mRNA expression of AGT were significantly reduced. To characterize the associated changes in intracellular signaling, the MAP kinase signaling cascade was evaluated, and alterations in p38 and JNK were identified. Furthermore, p38 and JNK were involved in the expression of AGT, suggesting the expression of TGF- β .

Conclusions

Reactive oxygen species stimulate mesangial cells to increase the secretion of AGT, but this increase is attenuated by the antioxidant isoflavones daidzein and equol.



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sciforum-122795: Targeting mitochondrial fission and extracellular mitochondria to ameliorate hepatic injury in metabolic dysfunction-associated fatty liver disease

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Background

Metabolic dysfunction-associated fatty liver disease (MASLD), formerly known as NAFLD, is a prevalent chronic liver condition characterized by steatosis, inflammation, and fibrotic progression. Emerging evidence suggests that dysregulated mitochondrial dynamics, particularly excessive mitochondrial fission, contribute to hepatic mitochondrial dysfunction and the release of pro-inflammatory extracellular mitochondria (exMito). However, the pathological role of this mechanism in MASLD remains incompletely understood.

Methods

To investigate the contribution of mitochondrial fission to MASLD progression, C57BL/6J mice were fed a high-fat diet (60% kcal from fat) for 12 weeks. Mitochondrial function, key regulators of fission (DRP1, p-DRP1(S616), FIS1, MID49, MID51), insulin signaling pathways, hepatic histopathology, and cytokine profiles were analyzed. Circulating exMito were isolated and characterized. A selective peptide inhibitor of DRP1/FIS1 interaction, P110, was administered to evaluate its therapeutic potential in restoring mitochondrial function and modulating exMito release.

Results

HFD-fed mice developed hallmark features of MASLD, including impaired glucose tolerance, hepatic macrovesicular/microvesicular steatosis, fibrosis, and elevated plasma alanine transaminase (ALT) and aspartate transaminase (AST) levels. These mice exhibited increased DRP1 activation, lipid peroxidation, and a marked shift in pro-inflammatory cytokines, accompanied by elevated levels of circulating exMito. Notably, treatment with P110 attenuated mitochondrial dysfunction reduced hepatic DRP1 activation, and improved glucose utilization. Liver histology showed decreased steatosis and collagen deposition, while cytokine profiling indicated a reversal of inflammatory responses. P110 also significantly reduced the release of exMito. Untargeted metabolomics revealed partial restoration of metabolic profiles, particularly in carbohydrate and tryptophan pathways.

Conclusion

Our findings highlight the pathological role of mitochondrial fission and extracellular mitochondria in MASLD. Pharmacological inhibition of DRP1/FIS1 interaction using P110 alleviates hepatic injury by restoring mitochondrial function, suppressing inflammatory exMito release, and improving systemic metabolic profiles. These results suggest that targeting mitochondrial dynamics may offer a promising therapeutic strategy for MASLD.



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sciforum-125464: The roles of chemical synapses in mouse taste bud cells

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Taste cells detect chemical compounds in the oral cavity and transmit these signals to gustatory nerve fibers. Taste cells are classified into four types: Type I, II, III, and IV. Among these, Type II cells are believed to mediate sweet, bitter, and umami tastes, while Type III cells are responsible for sour taste detection. Previous studies have demonstrated that only Type III cells form synaptic structures with nerve fibers; however, the function of these synapses remains unclear. In this study, the role of chemical synapses in taste cells was investigated using epithelial-specific *Snap25* knockout (*Snap25*-cKO) mice. Immunohistochemical analysis confirmed that SNAP25 expression was eliminated in the taste cells of *Snap25*-cKO mice. Additionally, the number of Type III taste cells was reduced in these mice. EdU tracing experiments indicated that while Type III cells may develop normally, their maintenance appears to be impaired in *Snap25*-cKO mice. Gustatory nerve recordings and behavioral analyses revealed that *Snap25*-cKO mice exhibited reduced sensitivity to sour compounds, but not to sweet, bitter, salty, or umami compounds. These findings suggest that chemical synapses in taste cells are essential for the maintenance of Type III cells and for the transmission of sour taste information from taste cells to gustatory nerve fibers.



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sciforum-124657: Vagal sensory neurons expressing oxytocin receptor regulate mental function via hypothalamic oxytocin neurons.

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Introduction

The increase in obesity and depression is a serious global issue. Oxytocin neurons in the hypothalamic paraventricular nucleus (PVH-OT neurons) regulate not only feeding but also mental functions. Therefore, PVH-OT neurons may serve as potential targets for ameliorating obesity and depression. However, no technology has yet been established to selectively activate PVH-OT neurons without side effects. We previously demonstrated that intraperitoneal (IP) injection of OT activates PVH-OT neurons via vagal afferent nerves expressing OT receptors, thereby ameliorating hyperphagic obesity in mice without aversive behaviors (Iwasaki Y et al., *BBRC* 2019). However, it remains unclear whether this peripheral-to-central OT coupling via vagal afferents also regulates mental functions. In the present study, we examined whether IP injection of OT could regulate mental functions, such as anxiety and sociability, through this pathway.

Methods & Results

A single IP injection of OT activated vagal sensory neurons via OT receptor and increased fluorescence of GCaMP6 expressing PVH-OT neurons, as measured by fiber photometry. Furthermore, IP OT significantly reduced anxiety-like behaviors in the elevated plus maze, and enhanced social behaviors in the three-chamber social interaction test. These OT-induced behavioral effects were attenuated by chemogenetic suppression of PVH-OT neurons using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), as well as by either intracerebroventricular or intraperitoneal administration of an OT receptor antagonist. Finally, as a pathway for peripheral-to-central signaling, subdiaphragmatic vagotomy abolished the psychotropic effects of OT.

Conclusion

We demonstrated that peripheral OT activates PVH-OT neurons via vagal afferent signaling, thereby regulating mental functions. Stimulating vagal afferents expressing OT receptors may represent a novel therapeutic approach for improving not only obesity but also psychiatric disorders.



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