# 6TH INTERNATIONAL CONFERENCE ON POLYAMINES: BIOCHEMICAL, PHYSIOLOGICAL AND CLINICAL PERSPECTIVES

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BIOCHEMICAL, PHYSIOLOGICAL AND CLINICAL PERSPECTIVES

# SCIENTIFIC PROGRAM AND ABSTRACTS

SAPIENZA University of Rome
and Tivoli (Rome), Italy
September 4-9, 2022

Under the auspices of

SAPIENZA
UNIVERSITÀ DI ROMA

IPFO
International Polyamines Foundation Otsus

Amine Pharma

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UNIVERSITY

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Kazuei Igarashi, Chiba University, Japan

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Prof. Enzo Agostinelli
Department of Sensory Organs-Faculty of Medicine and Dentistry
SAPIENZA University of Rome, Policlinico Umberto I
Viale del Policlinico 155, I-00161 Rome, Italy
Phone: +39 (0)6 49976857
e-mail: enzo.agostinelli@uniroma1.it

## CONFERENCE WEBSITE

https://2022.polyaminesfoundation.org

# WELCOME MESSAGE

Dear Attendees,

Following the tradition of successful Congresses held in several European countries, Japan, India, Turkey, South Africa, Russia, Brazil, Taiwan, USA and Italy, the Organizing Committee has the great honor to welcome you to Italy, to attend the 6th International Conference on Polyamines: Biochemical, Physiological and Clinical Perspectives at the SAPIENZA University of Rome and Tivoli, Rome, from September 4 to 9, 2022.

Tivoli is a small town very close to Rome. Most Latin authors link the founding of Tivoli with the figure of Evandrus. The town probably grew up in the 7th-8th century B.C. through the merging of small, surrounding villages, as can be seen from the articles found in the necropolises; standing on Monte Ripoli, Tivoli controls passage from the Aniene Valley on one side and, on the other, is connected with Rome along the navigable course of the river. After various events, it came under Roman rule in 338 B.C. and became a municipality; it acquired Roman citizenship in 87 B.C. and was included in the 4th Region: *Sabina et Samnium* in the Augustan division. Numerous imperial monuments, for the originality of their architectural solutions, deserve to be visited: among them, Hadrian's Villa and Villa d'Este. Therefore, we wish all of you a memorable time in Tivoli both scientifically and socially. On the scientific side, the Conference will cover a wide range of topics related to the biochemical and pathophysiological properties of polyamines. The scientific program will focus on the metabolism of polyamines, their essential role in cell growth and differentiation in animal, plant and microorganisms and, especially, their role in a variety of pathological conditions, with a special emphasis on cancer. The polyamine pathway as a target for drug development for cancer treatment or chemoprevention will also be a central theme.

We would like to acknowledge all the distinguished speakers who kindly agreed to act as a part of the lecturing team. The scientific program supported by excellent participants, both senior experts and enthusiastic newcomers, includes lectures and shorter oral poster communications, as well as sessions of poster exhibition spanning several research areas and many countries of origin. The main goal of the conference is to promote scientific exchange among research groups highly qualified in different but interrelated fields and to foster collaborative investigations in the areas represented in the Conference. The Organizers hope that the presentations at this meeting, gathering contributions from biochemists, pharmacologists, chemists, geneticists, molecular biologists and clinical scientists, will demonstrate the current state of knowledge on the physiological, biochemical and therapeutic actions of polyamines, providing a stimulus mainly for the new generation involved in the polyamine field.

To enjoy stay in Tivoli-Rome to the full, you should take an active part in the social events, including parties and sightseeing. Our information desk, at the Congress Center, will be at your service to help you with daily problems. We look forward to meeting you at the welcome reception!

We thank you all for contributing to the success of the Congress, and wish you a very pleasant time in Tivoli-Rome.

**Kazuei Igarashi**
Chiba University, Japan

**Enzo Agostinelli**
SAPIENZA University of Rome, Italy

# SCIENTIFIC PROGRAM

## SESSIONS

**1.** Polyamines in cell growth and differentiation

**2.** Eif5a and translation

**3.** Polyamines and physiology

**4.** Polyamines in human health: in cancer and other diseases. Therapeutic applications

**5.** Polyamines in nutrition and longevity

**6.** Polyamines and their analogs: chemistry and molecular pharmacology

**7.** Polyamines in plants and in biotechnological applications

**8.** Polyamines metabolism, transport and signal transduction

**9.** Polyamines metabolism in parasites and other microorganisms

# Sunday, September 4th

Grand Hotel Duca D'este, Tivoli

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| **1:30 pm – 5:30 pm** | | **Registration at the Grand Hotel Duca d'Este (Tivoli)** |
| **5:30 pm – 6:00 pm** | | **Opening Ceremony**<br>*E. Agostinelli, Rome, Italy*<br>*K. Igarashi, Chiba, Japan*<br>*U. Bachrach, Jerusalem, Israel* |
| **6:00 pm – 7:00 pm** | | **Opening Lecture**<br>*Session leaders*<br>*E. Agostinelli (SAPIENZA University of Rome, Italy)*<br>*U. Bachrach (Hebrew University, Israel)* |
| **6:00 pm- 7:00 pm** | [PL 01] | **K. Igarashi (Chiba University and Amine Pharma, Japan)**<br>Molecular mechanisms of cell and tissue toxicity caused by acrolein |
| **6:30 pm – 7:30 pm** | | **Registration at the Grand Hotel Duca d'Este (Tivoli)** |
| **7:30 pm – 10:30 pm** | | **Welcome dinner at the Grand Hotel Duca d'Este (Tivoli)** |

# Monday, September 5th

**9:00 am – 10:00 am** **Session 1: POLYAMINES IN CELL GROWTH AND DIFFERENTIATION**

*Session leader: T. Oka (Wakunaga Pharmaceutical Co.Ltd, Japan)*

**9:00 am – 9:30 am** [L 02] **A. Zabala Letona (Center for Cooperative Research in Biosciences, Spain)**
Emerging roles of polyamine metabolism in prostate cancer

**9:30 am – 10:00 am** [L 03] **M. Pujana Vaquerizo (Center for Cooperative Research in Biosciences, Spain)**
Analysis of the molecular and biological consequences of GC7 treatment in prostate cancer

**10:00 am – 12:40 pm** **Session 2: EIF5A AND TRANSLATION**

**10.00 am – 10:35 am** [PL 04] **A. Kaiser (University of Duisburg-Essen, Germany)**
Investigation of an allosteric deoxyhypusine synthase inhibitor in P.falciparum

**10:35 am – 11:05 am** **Coffee break**

**11:05 am – 11:40 am** [PL 05] **K.T. Wilson (Vanderbilt University Medical Center, USA)**
Protective Role of Spermidine and Hypusination in Colitis and Colitis-associated Colon Carcinogenesis

**11:40 am – 12:10 pm** [L 06] **G. Canettieri (Sapienza University of Rome, Italy)**
A novel interplay between polyamines, EIF5A and MYC in colorectal cancer

**12:10 am – 12:40 pm** [L 07] **A.Szepesi (University of Szeged, Hungary)**
Connection between GABA and hypusination process of Arabidopsis thaliana seedlings influencing polyamine catabolism.

**1:00 pm – 2:30 pm** **Lunch**

**3:30 pm – 5:00 pm** **Poster session and coffee break**

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| **5.00 pm – 6:35 pm** | | **Session 3: POLYAMINES AND PHYSIOLOGY** |
| | | *Session leader: K.Igarashi, (Chiba University and Amine Pharma, Japan)* |
| **5:00 pm – 5:35 pm** | [PL 08] | **K. Kashiwagi (Chiba institute of science, Japan)** ,<br>Regulation of gene expression through translational stimulation of histone modifying enzymes by polyamines |
| **5:35 pm – 6:05 pm** | [L 09], | **S. Coni (Sapienza University of Rome, Italy)**<br>Locomotor function in *Drosophila Melanogaster* is controlled by a CNBP/ODC/polyamines translational axis |
| **6:05 pm – 6:35 pm** | [L 10] | **T. Sieckmann (Institute of translational Physiology, Germany)**<br>Dysregulation of the polyamine system in favor of its catabolism is a common mechanism after kidney injury |
| **7:00 pm – 8:15 pm** | | **Dinner** |
| **8:30 pm – 12:00 am** | | **Rome by night** |

# Tuesday, September 6th

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| **9:00 am – 12:40 pm** | | **Session 4: POLYAMINES IN HUMAN HEALTH: IN CANCER AND OTHER DISEASES. THERAPEUTIC APPLICATIONS**<br>*Session leader: D. A. Spandidos (University of Crete, Greece)* |
| **9:00 am – 9:35 am** | [PL 11] | **S. Gilmour (Lankenau Institute for Medical Research, USA)**<br>Targeting the Immunomodulatory Effects of Polyamines in Cancer |
| **9:35 am – 10:05 am** | [L 12] | **M. Azfar (Laboratory of Cellular Transport Systems, Belgium)**<br>ATP13A3 in polyamine homeostasis and in the pathogenesis of Pulmonary Arterial Hypertension |
| **10:05 am – 10:35 am** | [L 13] | **T. Murray Stewart (Johns Hopkins University School of Medicine, Baltimore, USA)**<br>Polyamine metabolism in the pathology and treatment of Snyder-Robinson syndrome |
| **10:35 am – 11:05 am** | | **Coffee break** |
| **11:05 am-11:40 am** | [PL 14] | **A.S. Bachmann (Michigan State University, USA)**<br>DFMO Treatment of children with ODC-1 linked Bachmann-Bupp Syndrome: from discovery to clinic |
| **11:40 am – 12:10 pm** | [L 15] | **E. Agostinelli (Sapienza University of Rome, Italy)**<br>Enzymatic Spermine metabolites induce apoptosis associated with increase of p53, caspase-3 and miR-34a in both Neuroblastoma cells, SJNKP and the N-Myc-Amplified form IMR5 |
| **12:10 pm – 12:40 pm** | [L 16] | **G. Weiman (Children's Cancer Institute, Australia)**<br>Polyamine blockade inhibits cell growth and induces apoptosis in high-risk childhood leukaemia |
| **12:45 pm** | | **Photograph** |
| **1:00 pm – 2.30 pm** | | **Lunch** |
| **2:30 pm – 3.30 pm** | | **Meeting International Polyamines foundation** |

**3.30 pm – 4.00 pm** **Oral poster presentation**
*Session leader: P. Mariottini (University ROMA TRE, Rome, Italy)*

**4:00 pm – 5:00 pm** **Poster session and Coffee break**

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**5:00 pm – 6:50 pm** **Session 5: POLYAMINES IN NUTRITION AND LONGEVITY**
*Session leader: P. Mariottini (University ROMA TRE, Rome, Italy)*

**5:00 pm – 5:30 pm** [L 17] **M. Cervelli (*Department of Science, University Roma Tre, Rome, Italy)***
Spermidine treatment affects gene expression in mouse model of Amyotrophic Lateral Sclerosis

**5:30 pm – 6:00 pm** [L 18] **T. Uemura (Department of Forensic Medicine, Kyoto Prefectural University of Medicine, Japan)**
Aging associated change in polyamine metabolism

**6:00 pm – 6:30 pm** [L 19] **H-J Lin (Department of Bioscience and Biotechnology, Taiwan)**
Nutritional value of Spermidine for Strombidium sp. NTOU1, a marine ciliates and its potential on the ocean food chain and ecosystem

**6.30 pm – 6.50 pm** [L20] **D. A. Spandidos (University of Crete, Greece)**
Publishing in biomedical sciences

**7.30 pm** **Dinner**

# Wednesday, September 7th

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| **9:00 am – 12:05 pm** | | **Session 6 : POLYAMINES AND THEIR ANALOGS: AND MOLECULAR PHARMACOLOGY** |
| | | *Session leader: A. R. Khomutov (Russian Academy of Sciences, Russia)* |
| **9:00 am – 9:35 am** | [PL 21 ] | **U. Bachrach (Hebrew University-Hadassah Medical School, Israel)** The effect of substituted Amino-Alkyl-Anthraquinones on Eukaryotic cells |
| **9:35 am – 10:05 am** | [L 22] | **S.Tevosian (Department of Physiological Sciences, University of Florida, USA)** Mechanism of action for an alkylated polyamine analogue diethylnorspermine (DENSPM) in treating pheochromocytoma/paraganglioma |
| **10:05 am – 10:35 am** | [L 23] | **R. Ragno (Sapienza University of Rome, Italy)** Ligand-Based and structured-based studies on Polyamine analogues as Bovine Serum Amine Oxidase substrates |
| **10:35 am – 11:05 am** | | **Coffee Break** |
| **11:05 am – 11:35 am** | [L 24] | **O. Phanstiel (University of Central Florida, Orlando, USA)** Development of FUBP1 inhibitors to control cancer cell growth |
| **11:35 am – 12:05 pm** | [L25] | **M. Houdou (Laboratory of Cellular Transport Systems, Belgium)** Characterization of novel green fluorescent polyamine analogs for measuring polyamine transport of the P5B-type ATPases |
| **12.05 pm -1:30 pm** | | **Lunch** |
| **1:30 pm – 7:30 pm** | | **Sightseeing** |
| **8:00 pm – 10:30 pm** | | **Dinner** |

# Thursday, September 8th

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| **9:00 am – 12:30 pm** | | **Session 7 : POLYAMINES IN PLANTS AND IN BIOTECHNOLOGICAL APPLICATIONS**<br>*Session leader: F. Vianello (University of Padua, Italy)* |
| **9:00 am – 9:30 am** | [L 26] | **A. Mattoo (Sustainable Agricultural Systems Laboratory, USA)**<br>Comparative genomics assisted mapping of polyamine (PA) biosynthetic pathway in duckweed (Spirodela polyrhiza) genome reveals absence of ODC pathway and that PA synthesis genes are differentially regulated during growth, MeJA exposure and salt stress |
| **9:30 am – 10:00 am** | [L27] | **E. Sobieszczuk-Nowicka (Department of Plant Physiology, Poland)**<br>Unravelling the genetics of polyamine metabolism in barley for senescence-related crop improvement |
| **10.00 am – 10:30 am** | [L28] | **M. Arasimowicz-Jelonek (Department of Plant Ecophysiology, Poland)**<br>Genome-wide exploration of genetics of biogenic polyamines in barley |
| **10:30 am – 11:00 am** | | **Coffee break** |
| **11:00 am – 11:30 am** | [L29] | **A. Venerando (Department of Comparative Biomedicine and Food Science, Padua, Italy)**<br>Biotechnological and therapeutic applications of nanostructured hybrids of magnetic nanoparticles conjugated with amine oxidase |
| **11:30 am -12:30 pm** | | **Oral poster presentation**<br>*Session leaders: A.Toninello (University of Padua, Italy)* |
| **1:00 pm – 2:30 pm** | | **Lunch** |

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| **3:00 pm – 4:30 pm** | | **Session 8: POLYAMINES METABOLISM, TRANSPORT AND SIGNAL TRANSDUCTION**<br>*Session leader: A. Ilari (National Research Council of Italy (CNR), Italy)* |
| **3:00 pm – 3:30 pm** | [L30] | **N. Ignatenko (University of Arizona, Tucson, USA)**<br>Targeting polyamines metabolism to suppress SARS-CoV-2- related disease |

**3:30 pm – 4:00 pm** [L31] **S. van Veen (Laboratory of Cellular Transport Systems Department of Cellular and Molecular Medicine, Belgium)**
A novel class of polyamine transporters in health and disease

**4.00 pm – 4.30 pm** [L32] **S. Vrijsen (Laboratory of Cellular Transport Systems, Department of Cellular and Molecular Medicine, Belgium)**
Elucidating the role of the lysosomal polyamine exporter ATP13A2 in mitochondrial-lysosomal interplay

**4:30 pm – 5:30 pm** **Poster session and coffee break**

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**5:30 pm – 6:30 pm** **Session 9: POLYAMINES METABOLISM IN PARASITES AND OTHER MICROORGANISMS**

**5:30 pm – 6:00 pm** [L 33] **S. Fujiwara (Department of Bioscience, Kwansei-Gakuin University, Japan)**
Identification of unique arginine decarboxylase involved in low pH dependent agmatine production in solid-state cultivated Aspergillus oryzae,

**6:00 pm – 6:30 pm** [L34] **G. Colotti (National research council, CNR, Italy)**
Optimization of potent and Specific Trypanothione Reductase Inhibitors: a structure-based drug discovery approach

**6:30 pm – 6:50 pm** *Concluding Remarks: E. Agostinelli (SAPIENZA University of Rome, Italy)*

**8:00 pm** **Gala Dinner**

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# Friday, September 9th

**9:00 o' clock am** **Departure to Fiumicino Airport and Termini Station**

# SCIENTIFIC PROGRAM
# ORAL POSTER PRESENTATIONS

## Tuesday, September 6th

**3:30 pm – 4:00 pm** *Session leader: P. Mariottini (University ROMA TRE, Rome, Italy)*

**3.30 pm – 3:45 pm** [P01] **T. Tahara (Sapienza University of Rome, Italy)**
Anti-cancer effect of spermidine inducing autophagy-dependent cell death

**3:45 pm - 4:00 pm** [P02] **S.van Veen (Laboratory of Cellular Transport System, Belgium)**
Quest for the transported of substrate of ATP13A4, a putative polyamine transported linked to neurodevelopmental disorders

## Thursday, September 8th

**11:30 am – 12:30 pm** *Session leader: A. Toninello (University of Padua, Italy)*

**11:30 am – 11:45 am** [P03] **O.Phanstiel (University of Central Florida, USA)**
Development of LAT- 1 efflux agonists to control pancreatic cancer Cell growth

**11:45 am – 12:00 pm** [P 04] **G. Weiman (Children's Cancer Institute, Australia)**
Polyamine transporter ATP13A3 is a novel therapeutic target in neuroblastoma

**12:00 pm – 12:15 pm** [P05] **A. R. Khomutov (Russian Academy of Sciences, Russia)**
Phosphorus analogues of AdoMet and AdoHCy: synthesis and interaction with AdoMet decarboxylase and DNA methyltransferase Dnmt3a

**12:15 pm – 12:30 pm** [P06] **G. Rilievo (University of Padua, Italy)**
Functional nanocarrier for bovine serum amine oxidase

# LECTURES

**[PL01]** Kazuei Igarashi, Takeshi Uemura, Akihiko Sakamoto, Yusuke Terui, and Keiko Kashiwagi
**Molecular mechanisms of cell and tissue toxicity caused by acrolein**

**[L02]** Amaia Zabala-Letona, Mikel Pujana-Vaquerizo, Onintza Carlevaris, Amaia Ercilla, Maider Fagoaga, Encarnación Pérez, Lorea Valcarcel-Jimenez, Amaia Arruabarrena-Aristorena, Natalia Martin-Martin, Nerea Sahuquillo, Isabel Mendizabal, Edurne Berra, Christian Frezza, Arkaitz Carracedo
**Emerging roles of polyamine metabolism in prostate cancer**

**[L03]** Mikel Pujana-Vaquerizo, Lorea Valcarcel-Jimenez, Amaia Ercilla, Ming Yang, Maider Fagoaga, Onintza Carlevaris, Amaia Arruabarrena-Aristorena, Natalia Martin-Martin, Marco Piva, Nerea Sahuquillo, Cristina Viera, Edurne Berra, Christian Frezza, Amaia Zabala-letona, Arkaitz Carracedo.
**Analysis of the molecular and biological consequences of GC7 treatment in prostate cancer**

**[PL04]** Aiyada Aroonsri, Chayaphat Wongsombat, Philip Shaw, Siegrid Franke, Jude Przyborski and Annette Kaiser
**Investigation of an allosteric deoxyhypusine synthase inhibitor in *P. falciparum***

**[PL05]** Alain P. Gobert, Thaddeus M. Smith, Yvonne L. Latour, Mohammad Asim, Daniel P. Barry, Margaret M. Allaman, Kamery J. Williams, Kara M. McNamara, Alberto G. Delgado, Sarah P. Short, Raghavendra G. Mirmira, Kristie L. Rose, Kevin L. Schey, Shilin Zhao, M. Blanca Piazuelo, M. Kay Washington, Lori A. Coburn, and Keith T. Wilson
**Protective Role of Spermidine and Hypusination in Colitis and Colitis-associated Colon Carcinogenesis**

**[L06]** Gianluca Canettieri
**A novel interplay between polyamines, EIF5A and MYC in colorectal cancer**

**[L07]** Ali Mohamed Ali, Lilla Sípos, László Bakacsy, Laura Zsigmond, Ágnes Szepesi
**Connection between GABA and hypusination process of *Arabidopsis thaliana* seedlings influencing polyamine catabolism**

**[PL08]** Keiko Kashiwagi, Akihiko Sakamoto, Yusuke Terui and Kazuei Igarashi
**Regulation of gene expression through translational stimulation of histone modifying enzymes by polyamines**

**[L09]** Sonia Coni, Marta Marzullo Federica Falconio Rosa Bordone, Enzo Agostinelli, Laura Ciapponi, Gianluca Canettieri
**Locomotor function in *Drosophila Melanogaster* is controlled by a CNBP/ODC/polyamines translational axis**

**[L10]** T. Sieckmann, N. Ögel, S. Kelterborn, F. Boivin, G. Schley, M. Fähling, M. I. Ashraf, M. Reichel, E. Vigolo, A. Hartner, F. Knauf, C. Rosenberger, F. Aigner, K. Schmidt-Ott, H. Scholz and K. M. Kirschner
**Dysregulation of the polyamine system in favor of its catabolism is a common mechanism after kidney injury**

**[PL11]** Eric Alexander, Sharon Shania, Olivia El Naggar, Erin Fahey, Otto Phanstiel, and Susan Gilmour
**Targeting the Immunomodulatory Effects of Polyamines in Cancer**

**[L12]** Mujahid Azfar, Nathalie Jacobs, Jialin Chen, Bin Liu, Norin Hamouda, Shaun Martin, Sarah Van Veen, Nicholas Morrell, Peter Vangheluwe
**ATP13A3 in polyamine homeostasis and in the pathogenesis of Pulmonary Arterial Hypertension**

**[L13]** Tracy Murray Stewart, Jackson R. Foley, and Robert A. Casero, Jr.
**Polyamine metabolism in the pathology and treatment of Snyder-Robinson syndrome**

**[PL14]** Andre S. Bachmann, Chad R. Schultz, Elizabeth A. Vansickle, Julianne Michael, Jeremy W. Prokop, Surender Rajasekaran, Caleb P. Bupp
**DFMO Treatment of Children with ODC1-linked Bachmann-Bupp Syndrome: From Discovery to Clinic**

**[L15]** Enzo Agostinelli
**Spermine metabolite, hydrogen peroxide and aldehyde, cause apoptosis in neuroblastoma cells associated with increase of p53, Caspase-3 and miR-34a**

**[L16]** Weiman Gao, Mawar Karsa, Lin Xiao, Ruby Pandher, Emma Ronca, Angelika Bongers, Murray D. Norris, Michelle Haber, and Klaartje Somers
**Polyamine blockade inhibits cell growth and induces apoptosis in high-risk childhood leukaemia**

**[L17]** C. Fiorucci, M. N. Rossi, O. Carletta, S. Giuliani, M. Terricola, S. Scaricamazza, F. Berardinelli, A. Ferri, C. Valle, Moreno S., P. Mariottini, Manuela Cervelli
**Spermidine treatment affects gene expression in mouse model of Amyotrophic Lateral Sclerosis**

**[L18]** Takeshi Uemura, Yuka Yokota, Miki Matsunaga, Yoshihisa Akasaka, Koichi Takao, Hiroshi Ikegaya, Takemitsu Furuchi
**Aging associated change in polyamine metabolism**

**[L19]** Bo-Ying, Su, Hung-Yun Lin and Han-Jia Lin
**Nutritional value of Spermidine for *Strombidium sp.* NTOU1, a marine ciliates, and its potential impact on the ocean food chain and ecosystems**

**[L20]** Demetrios A. Spandidos
**Publishing in biomedical sciences**

**[PL21]** Uriel Bachrach
**The effect of subtituted amino-alkyl-anthraquinones on eukaryotic cells**

**[L22]** Sergei G Tevosian, Abdel A. Alli, Niharika Bala, Raymond Bergeron, Hans K Ghayee, Heather M. Hatch, Yiling Xu
**Mechanism of action for an alkylated polyamine analogue diethylnorspermine (DENSPM) in treating pheochromocytoma/paraganglioma**

**[L23]** Rino Ragno, Eleonora Proia, Antonini Lorenzo, Lavinia Rutigliano, Alessandro Montella, Tomoaki Tahara, Giancarlo Altissimi, Alessio Ragno, Roberto Capobianco, Gianluca Canettieri, and Enzo Agostinelli
**Ligand-Based and Structure-Based Studies on Polyamine Analogues as Bovine Serum Amine Oxidase Substrates**

**[L24]** Holly Moots, Aiste Dobrovolskaite, Mukund Tantak, Alex Bunea, Christopher Polera, Chloe Robinson and Otto Phanstiel.
**Development of FUBP1 inhibitors to control cancer cell growth**

**[L25]** Marine Houdou, Nathalie Jacobs, Jonathan Coene, Roeland Vanhoutte, Mujahid Azfar, Veronique Daniëls, Steven Verhelst and Peter Vangheluwe
**Characterization of novel green, fluorescent polyamine analogs for measuring polyamine transport of the P5B-type ATPases**

**[L26]** Rakesh K. Upadhyay, Jonathan Shao, and Autar K. Mattoo
**Comparative genomics assisted mapping of polyamine (PA) biosynthetic pathway in duckweed (*Spirodela polyrhiza*) genome reveals absence of ODC pathway and that PA synthesis genes are differentially regulated during growth, MeJA exposure and salt stress**

**[L27]** Umesh Kumar Tanwar, Ewelina Stolarska, Ewelina Paluch-Lubawa, Autar K. Mattoo, Magdalena Arasimowicz-Jelonek, Ewa Sobieszczuk-Nowicka
**Unraveling the genetics of polyamine metabolism in barley for senescence-related crop improvement**

**[L28]** Umesh Kumar Tanwar, Ewelina Stolarska, Ewelina Paluch-Lubawa, Autar K. Mattoo, Ewa Sobieszczuk-Nowicka, Magdalena Arasimowicz-Jelonek
**Genome-wide exploration of the genetics of biogenic polyamines in barley**

**[L29]** Andrea Venerando, Yuta Kanamori, Alberto Macone, Massimiliano Magro, Enzo Agostinelli, Fabio Vianello
**Biotechnological and therapeutic applications of nanostructured hybrids of magnetic nanoparticles conjugated with amine oxidase**

**[L30]** Natalia A. Ignatenko, Hien Trinh, April Wagner, David G. Besselsen
**Targeting polyamine metabolism to suppress SARS-CoV-2-related disease**

**[L31]** Sarah van Veen, Norin Nabil Hamouda, Mujahid Azfar, Stephanie Vrijsen, Jianmin Si, Chris Van Den Haute, Veerle Baekelandt, Jan Eggermont, Shaun Martin, Peter Vangheluwe
**A novel class of polyamine transporters in health and disease**

**[L32]** Stephanie Vrijsen, Chris Van den Haute, Chase Chen, Yu Chen, Ellen Sidransky, Veerle Baekelandt, Peter Vangheluwe
**Elucidating the role of the lysosomal polyamine exporter ATP13A2 in mitochondrial-lysosomal interplay**

**[L33]** Yui Murakami, Wakao Fukuda, Naoki Akasaka and Shinsuke Fujiwara
**Identification of unique arginine decarboxylase involved in low pH dependent agmatine production in solid-state cultivated *Aspergillus oryzae***

**[L34]** Gianni Colotti, Theo Battista, Stefano Federico, Simone Brogi, Luca Pozzetti, Stefania Butini, Anna Ramunno, Eleonora Fiorentino, Stefania Orsini, Trentina Di Muccio, Annarita Fiorillo, Cécile Exertier, Sandra Gemma, Giuseppe Campiani Andrea Ilari
**Optimization of Potent and Specific Trypanothione Reductase Inhibitors: A Structure-Based Drug Discovery Approach**

[PL01]

## Molecular mechanisms of cell and tissue toxicity caused by acrolein

Kazuei Igarashi[1 2], Takeshi Uemura[1 2], Akihiko Sakamoto[3], Yusuke Terui[3], Keiko Kashiwagi[3]

[1] *Amine Pharma Research Institute, Innovation Plaza at Chiba University, Chiba, Japan*

[2] *Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan*

[3] *Faculty of Pharmacy, Chiba Institute of Science, Choshi, Chiba, Japan*

*E-mail: iga16077@gmail.com*

Acrolein ($CH_2$=CH-CHO), an unsaturated aldehyde produced from spermine, is one of the major substances causing oxidative stress. Acrolein has been found to be more toxic than reactive oxygen species ($H_2O_2$ and •OH), and it can be easily conjugated with proteins, bringing about changes in nature of the proteins. Accordingly, the molecular mechanisms of acrolein toxicity and tissue damage elicited by acrolein were investigated. It was found that GAPDH (glyceraldehyde-3-phosphate dehydrogenase), cytoskeleton proteins such as vimentin, actin, α- and β-tubulin proteins, and apolipoprotein B-100 (ApoB100) in LDL are strongly damaged by acrolein conjugation. In contrast, activities of matrix metalloproteinase-9 (MMP-9) and proheparanase (proHPSE) are stimulated, and antibody recognizing abilities of imumunoglobulins are modified by acrolein conjugation resulting in aggravation of diseases. The functional changes of these proteins by acrolein are clarified at the molecular levels. This time, the molecular mechanisms of tissue damage caused by acrolein through its conjugation with GAPDH, α- and β-tubulin proteins, ApoB100 and MMP-9 have been clarified. These findings confirmed the idea that acrolein is the major substance causing the tissue damage in the elderly.

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## SESSION 1

*Polyamines in cell growth and differentiation*

[L02]

## Emerging roles of polyamine metabolism in prostate cancer

Amaia Zabala-Letona[1 4*], Mikel Pujana-Vaquerizo[1*], Onintza Carlevaris[1], Amaia Ercilla[1 4], Maider Fagoaga[1], Encarnación Pérez[1], Lorea Valcarcel-Jimenez[2], Amaia Arruabarrena-Aristorena[1 3], Natalia Martin-Martin[1 3 4], Nerea Sahuquillo[1], Isabel Mendizabal [1], Edurne Berra[1 4], Christian Frezza[2], Arkaitz Carracedo[1 3 4 5 6]

[1] *Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, Building 801A, 48160 Derio, Spain*

[2] *CECAD Research Centre, University of Cologne, Joseph-Stelzmann-Str. 26, 50931 Cologne, 11 Germany*

[3] *Traslational prostate cancer Research lab, CIC bioGUNE-Basurto, Biocruces Bizkaia Health Research Institute, Spain*

[4] *Centro de Investigación Biomédica En Red de Cáncer (CIBERONC), 28029 Madrid, Spain*

[5] *IKERBASQUE, Basque Foundation for Science, 48009 Bilbao, Spain*

[6] *Biochemistry and Molecular Biology Department, University of the Basque Country (UPV/EHU), P.O. Box 644, E-48080 Bilbao, Spain*

**Equal contribution*

*Email: azabala@cicbiogune.es*

Prostate cancer (PCa) is among the most frequent cancers in men. Although it has been largely studied and many therapeutic approaches have been tested preclinical and clinically, it continues to cause a large number of deaths in developed societies[1]. Importantly, the majority of prostate cancers exhibit hyperactivation of the PI3K-mTORC1 pathway, which regulates cell growth, survival and metabolism[2]. In the last years and taking advantage of an integrative metabolomic approach we have described the importance of polyamine metabolism in PCa progression and we showed that mechanistic target of rapamycin complex 1 (mTORC1) regulates polyamine dynamics, a metabolic route that is essential for oncogenicity[3]. Despite intensive research, the molecular effectors and biological consequences of polyamines remain largely elusive and this leads to failed therapeutic approaches in cancer. We have undertaken a multiomics strategy and describe a survival phenotype in PCa cells upon polyamine deprivation therapies that lead us to open new therapeutic combinatorial approaches in PCa. Overall we have uncovered unprecedented processes under the control of polyamines that lead us to propose new functions for these metabolites in heath and disease.

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[L03]

## Analysis of the molecular and biological consequences of GC7 treatment in prostate cancer

Mikel Pujana-Vaquerizo[1], Lorea Valcarcel-Jimenez[2], Amaia Ercilla[1 4], Ming Yang[2], Maider Fagoaga[1], Onintza Carlevaris[1], Amaia Arruabarrena-Aristorena[1 3], Natalia Martin-Martin[1 3 4], Marco Piva[1 3], Nerea Sahuquillo[1], Cristina Viera[1 4], Edurne Berra[1 4], Christian Frezza[2], Amaia Zabala-letona[1 4], Arkaitz Carracedo[1 3 4 5 6]

[1] *Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Bizkaia Technology Park, Building 801A, 48160 Derio, Spain*

[2] *CECAD Research Centre, University of Cologne, Joseph-Stelzmann-Str. 26, 50931 Cologne, 11 Germany*

[3] *Traslational prostate cancer Research lab, CIC bioGUNE-Basurto, Biocruces Bizkaia Health Research Institute, Spain*

[4] *Centro de Investigación Biomédica En Red de Cáncer (CIBERONC), 28029 Madrid, Spain*

[5] *IKERBASQUE, Basque Foundation for Science, 48009 Bilbao, Spain*

[6] *Biochemistry and Molecular Biology Department, University of the Basque Country (UPV/EHU), P.O. Box 644, E-48080 Bilbao, Spain*

*Email: mpujana@cicbiogune.es*

The study of tumor metabolism has uncovered a new therapeutic window for the treatment of some cancer types. In such paradigm, cell signaling and metabolic reprogramming can act in tandem for the benefit of the cancer cell[1]. Prostate cancer (PCa), the second leading cause of cancer death in men, is a clear example of this convergence. Previous studies conducted in our lab have shown how polyamine biosynthesis is elevated in PCa with profound implications in the progression of the disease[2]a metabolic route that is essential for oncogenicity. By using integrative metabolomics in a mouse model and human biopsies of prostate cancer, we identify alterations in tumours affecting the production of decarboxylated S-adenosylmethionine (dcSAM. However, polyamine deprivation therapy has failed in patients in the last years and currently, in spite of the conclusive data about the contribution of polyamines to cancer biology, their molecular effectors remain obscure. One potential mechanism involves the hypusination, an intermediate reaction coming from spermidine, which consists of a posttranslational modification of EIF5A molecule that impacts on proteins translation elongation[3]eukaryotic translation initiation factor 5A (eIF-5A, old terminology, eIF-4D. From a therapeutic perspective, the spermidine analog GC7 has emerged as the main hypusination inhibitor used in the clinics[4]. However, attributing specific effects of GC7 to hypusination inhibition or to polyamine displacement by analogy has become a huge challenge. In this work, we analyze the role of GC7 treatment in DU145 prostate cancer cell line to shed light on the molecular mechanism underlying some reported and no reported effects.

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## SESSION 2

*Eif5a and translation*

[PL04]

## Investigation of an allosteric deoxyhypusine synthase inhibitor in *P. falciparum*

Aiyada Aroonsri[1], Chayaphat Wongsombat[1], Philip Shaw[1],
Siegrid Franke[2], Jude Przyborski[2], Annette Kaiser[3*]

[1] *National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand.*

[2] *Interdisziplinäres Forschungszentrum IFZ, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany.*

[3] *Medical Research Centre, University of Duisburg-Essen, Hufelandstrasse 55, 45147 Essen, Germany.*

*Email:kaiser@microbiology-bonn.de*

The treatment of a variety of protozoal infections, in particular, those causing disabling human diseases is still hampered by a lack of drugs or increasing resistance to registered drugs. However, in *recent* years remarkable progress has been achieved to combat neglected, tropical diseases by sequencing the parasites' genomes or validation of new targets in the parasites by novel genetic manipulation techniques leading to loss of function. The novel amino acid hypusine is a posttranslational modification (PTM) that occurs in eukaryotic initiation factor 5A (EIF5A) at a specific lysine residue. This modification occurs by two steps catalyzed by deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH) enzymes. DHS from *Plasmodium* has been validated as a druggable target by small molecules and reverse genetics. Recently, the synthesis of a series of human DHS inhibitors led to 6-bromo-N-(1H-indol-4yl)-1-benzothiophene-2-carboxamide, a potent allosteric inhibitor with an $IC_{50}$ value of 0.062 µM. We investigated this allosteric DHS inhibitor in *Plasmodium. In vitro P. falciparum* growth assays showed weak inhibition activity, with $IC_{50}$ values of 46,1 µM for the Dd2 strain and 51.5 µM for the 3D7 strain, respectively. The antimalarial activity could not be attributed to the targeting of the *Pfdhs* gene, as shown by chemogenomic profiling with transgenically modified *P. falciparum* lines. Moreover, in dose-dependent enzymatic assays with purified recombinant *P. falciparum* DHS protein, only 45% inhibition was observed at an inhibitor dose of 0.4 µM. These data are in agreement with a homology modeled *Pf*DHS suggesting significant structural differences in the allosteric site between the human and parasite enzymes. Virtual screening of the Allosteric database identified candidate ligands binding to novel binding pockets identified in *P. falciparum* DHS, which might foster the development of parasite-specific inhibitors.

Keywords: chemogenomic profiling, Hypusine, Bromobenzothiophene, Deoxyhypusine Synthase, *glmS* riboswitch, allosteric inhibitor

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[PL05]

## Protective Role of Spermidine and Hypusination in Colitis and Colitis-associated Colon Carcinogenesis

Alain P. Gobert[1 2], Thaddeus M. Smith[1], Yvonne L. Latour[1 4], Mohammad Asim[1], Daniel P. Barry[1], Margaret M. Allaman[1], Kamery J. Williams[1], Kara M. McNamara[1 3], Alberto G. Delgado[1], Sarah P. Short[1 2], Raghavendra G. Mirmira[5], Kristie L. Rose[6], Kevin L. Schey[6], Shilin Zhao[7], M. Blanca Piazuelo[1 2], M. Kay Washington[2 4], Lori A. Coburn[1 2 3 8], Keith T. Wilson[1 2 3 4 8]

[1] *Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine*

[2] *Center for Mucosal Inflammation and Cancer*

[3] *Program in Cancer Biology*

[4] *Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA*

[5] *Translational Research Center, Department of Medicine, The University of Chicago, Chicago, IL, USA*

[6] *Department of Biochemistry, Mass Spectrometry Research Center, Vanderbilt University School of Medicine, Nashville, TN, USA*

[7] *Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN 37232, USA*

[8] *Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN, USA*

Ulcerative colitis (UC) and Crohn's disease (CD) are the two forms of inflammatory bowel disease. They are common public health problems, and they can lead to colitis-associated carcinogenesis (CAC). We recently reported (*Gastroenterology*, 2022) that the polyamine spermidine reduces colitis and CAC in mouse models. Spermidine is converted by deoxyhypusine synthase (DHPS) into hypusine, which is conjugated to the eukaryotic translation initiation factor 5A (EIF5A), a process termed hypusination. Hypusinated EIF5A (EIF5A$^{Hyp}$) binds specific mRNAs and initiates translation. The role of hypusination in the gut is unknown. Our aim was to determine the role of hypusination in colitis and CAC. When compared to normal control tissues, we found reduced levels of *DHPS* mRNA in UC samples from our facility and in 7 UC and 3 CD publicly available transcriptional datasets. We also detected reduced protein levels of DHPS and EIF5A$^{Hyp}$ in colonic epithelial cells of patients with UC and CD by immunofluorescence on tissue microarrays. We generated mice with intestinal epithelial-specific deficiency of *Dhps* by crossing *Dhps$^{fl/fl}$* to *Vil1$^{cre/+}$* mice, yielding *Dhps$^{Depi}$* mice, confirmed by knockdown of *Dhps* mRNA and DHPS protein, and loss of EIF5A$^{Hyp}$. *Dhps$^{Depi}$* mice develop spontaneous colon hyperplasia, epithelial proliferation, crypt distortion, and inflammation. Compared to littermate *Dhps$^{fl/fl}$* controls, these mice are highly susceptible to experimental colitis and show exacerbated colon tumorigenesis when treated with the carcinogen azoxymethane. Proteomic analysis on colonic epithelial cells from untreated mice shows that compared to *Dhps$^{fl/fl}$* controls, *Dhps$^{Depi}$* mice exhibit attenuated levels of numerous enzymes involved in aldehyde detoxification, including glutathione S-transferases and aldehyde dehydrogenases, and increased proteins associated with carcinogenesis and immune activation. RNA sequencing also implicated a pro-carcinogenic state in *Dhps$^{Depi}$* mice. The hypusination-deficient mice exhibit increased levels of nuclear aldehyde adducts in the colon and their treatment with a scavenger of reactive aldehydes attenuates their exacerbated colitis. Thus, hypusination in epithelial cells holds potential for treatment of colitis and prevention of CAC.

[L06]

## A novel interplay between polyamines, EIF5A and MYC in colorectal cancer

Gianluca Canettieri

*Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena 291, 00161 Rome, International Polyamines Foundation-ONLUS, Via del Forte Tiburtino 98, 00159 Rome, Italy.*

Intestinal tumorigenesis is in most cases associated to increased expression of ornithine decarboxylase (ODC), the first rate-limiting enzyme of polyamine metabolism and a transcriptional target of MYC. ODC and polyamines are upregulated in many CRCs and their inhibition is considered a potential therapeutic avenue. DFMO (Difluoromethylornithine) an irreversible inhibitor of ODC has been shown to limit intestinal tumorigenesis in preclinical models and patients, raising interest in this drug *(Gerner E. 2018).* However, chronic ODC inhibition eventually leads to resistance, due to increased uptake of extracellular polyamines, indicating that novel strategies to stably reduce intracellular polyamine content and/or function may be preferable. A key downstream regulator of polyamine function is EIF5A, a translation factor activated by hypusination, a unique post-translational modification catalyzed by deoxyhypusine synthetase (DHPS) and deoxyhypusine hydroxylase (DOHH), which conjugate spermidine to the Lys-50 of EIF5A. Activity of DHPS enzyme can be inhibited by GC7 (N1-guanyl-1,7-diaminoheptane), an analogous of spermidine. Hypusinated EIF5A (hyp-EIF5A) regulates key cellular processes such as autophagy, energy metabolism and plays a role in cancer *(Casero R.A. ey al 2018, Park MH et al 2018),* but the effects of its inhibition in preclinical cancer models, and the specific translational targets are still poorly understood. We have discovered that hyp-EIF5A promotes growth of colorectal cancer (CRC) cells by directly regulating MYC translation (*Coni S* et al, 2020). By alleviating ribosome stalling at specific pausing sites in the coding sequence, EIF5A enhances MYC elongation in response to elevations in polyamine content. Thus, since MYC overexpression promotes an increase of ODC transcription and polyamine content, this suggests the existence of a positive MYC-ODC-EIF5A feedback loop that gets amplified in cancer and can be interrupted by inhibiting EIF5A function. Indeed, we demonstrate that inhibition of EIF5A-hyp formation with the DHPS inhibitor GC7 or through lentiviral-mediated knockdown of DHPS or EIF5A reduces the growth of several CRC cells, and significantly reduces polyp size in $APC^{Min/+}$ mice, a model of human Familial Adenomatous Polyposis (FAP). Remarkably, while treatment with GC7 and DFMO alone reduces tumor cell proliferation through a cytostatic mechanism, the combination of the two drugs causes apoptosis and a significant reduction of tumor growth in mouse models, by convergent effects on MYC translation. Together, our data illustrate a novel mechanism whereby the tumor-promoting properties of polyamines and hyp-EIF5A are linked to their ability to synergistically regulate MYC translation, a process that could be targeted by combinations of ODC and DHPS inhibitors.

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Cell Death Dis. Dec 10;11(12):1045. (2020)

[L07]

## Connection between GABA and hypusination process of *Arabidopsis thaliana* seedlings influencing polyamine catabolism

Ali Mohamed Ali[1], Lilla Sípos[1], László Bakacsy[1], Laura Zsigmond[2], Ágnes Szepesi[1]*

[1] *University of Szeged, Szeged, Hungary*

[2] *Biological Research Centre, ELKH, Szeged, Hungary*

**e-mail: szepesia@bio.u-szeged.hu*

Gamma-aminobutyric acid (GABA) functions as a signalling molecule and a metabolite and has been implicated in playing a critical role in plant salinity stress tolerance. Hypusination, a spermidine-dependent posttranslational modification of eIF5A translation factor (Pálfi et al. 2021) could be involved in salt stress responses, however our knowledge about connection between GABA and hypusination is limited. In this study, we determined the effect of exogenous GABA on Arabidopsis salt stress tolerance by examining morphological and physiological parameters and the interactions between GABA, polyamines, and the hypusination process in salt stress tolerance. Two weeks-old Arabidopsis seedlings were subjected to 1 mM GABA and 100 mM NaCl treatments. NaCl stress negatively affected the phenotypic appearance of the seedlings and significantly decreased the physiological parameters and expression levels of genes coding *AtDHS*, *AtDOHH* and the three *AteIF5A* isoforms. Under control conditions, exogenous GABA significantly improved the physiological parameters and expression levels of genes involved in hypusination. However, exogenous GABA significantly increased primary root length, Put, *AtDOHH* and *AteIF5A-1* expression under salt stress conditions, but significantly decreased Spm, total PAs, DAO and PAO activity and *AtDHS* expression. At the same time, there was no significant change in fresh weight, protein content, chlorophyll content, Spd, $H_2O_2$ and $O_2^-$ production, and *AteIF5A-2* and *AteIF5A-3* expression. Our results showed that exogenous GABA modulated polyamine metabolism reducing PAs degradation and increasing expression level of genes involved in the hypusination process. In conclusion, exogenous GABA is suggested to be involved in the Arabidopsis hypusination process and contribute to salt stress tolerance. This work was supported by OTKA FK129061 grant.

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## SESSION 3

*Polyamines and physiology*

[PL08]

## Regulation of gene expression through translational stimulation of histone modifying enzymes by polyamines

Keiko Kashiwagi[1], Akihiko Sakamoto[1], Yusuke Terui[1], Kazuei Igarashi[2 3]

[1] *Faculty of Pharmacy, Chiba Institute of Science, Choshi, Chiba, Japan*

[2] *Amine Pharma Research Institute, Innovation Plaza at Chiba University, Chiba, Japan*

[2] *Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan*

*E-mail: kkashiwagi@cis.ac.jp*

Since polyamines exist mainly as polyamine-RNA complexes, polyamines enhance translation of several proteins, which are important for cell growth and viability. We have proposed that a set of genes whose expression is enhanced by polyamines at the level of translation can be classified as a "polyamine modulon", and thus far identified 20 different genes in *Escherichia coli* and 11 different genes in eukaryotes as component of polyamine modulon. In mammalian cells, to identify polyamine modulon, polyamine-reduced cells were prepared using α-difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase, and proteins in cells were compared between normal and polyamine-reduced cells. In this study, we examined the effects of polyamines on histone modification: acetylation and methylation. Histone acetylation usually causes transcriptional activation, whereas histone methylation causes transcriptional activation and repression depending on the site of lysine residue. Total activities of histone acetyltransferases in nuclear and cytosol fractions in FM3A cells were significantly higher in control cells than DFMO-treated, i.e. polyamine-reduced cells. Although protein levels of histones H3 and H4 did not alter by DFMO-treatment, acetylation levels were about 2-fold higher in control cells than polyamine-reduced cells. When the protein levels of 13 kinds of histone acetyl transferases were compared, those of Gcn5 and Hat1 were 3-fold higher in control than polyamine-reduced cells, whereas mRNA levels did not alter. So, the genes for Gcn5 and Hat1 were identified as members of polyamine modulon. As for the mechanism of stimulation of Gcn5 synthesis by polyamines, it was found that polyamines enhance interaction of Gcn5 mRNA with a micro RNA, which enhances translation by interaction with mRNA. Because HAT1 mRNA has a short 5'-untranslated region, polyamines may enhance initiation complex formation directly on this mRNA. Because the level of methylation of lysine residues (K4, K9, K27, K36 and K79) in histone H3 was increased in DFMO-treated, polyamine reduced FM3A cells, protein expression levels of 9 histone demethylases were compared in control and DFMO-treated cells. The protein levels of three histone demethylases, JARID1C, JMJD2A, and UTX were 2- to 3-fold higher in control cells than in DFMO-treated cells. Since mRNA levels of these proteins were not changed in control and DFMO-treated cells, genes for JARID1C, JMJD2A, and UTX were identified as members of polyamine modulon.

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[L09]

## Locomotor function in *Drosophila Melanogaster* is controlled by a CNBP/ODC/polyamines translational axis

Sonia Coni[*1], Marta Marzullo[*2], Federica Falconio[*2 3], Rosa Bordone[1], Enzo Agostinelli[4 5], Laura Ciapponi[2], Gianluca Canettieri[1 5 6]

[1] *Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy;*

[2] *Department of Biology and Biotechnologies, Sapienza University of Rome, Rome, Italy;*

[3] *Department of Life Sciences Imperial College London South Kensington campus, London, United Kingdom;*

[4] *Department of Sensory Organs, Sapienza University of Rome, Policlinico Umberto I, Rome, Italy;*

[5] *International Polyamines Foundation 'ETS-ONLUS', Rome, Italy;*

[6] *Pasteur Institute, Fondazione Cenci-Bolognetti, Rome, Italy*

*sonia.coni@uniroma1.it*

CNBP (ZNF9) is a conserved CCHC-type zinc finger *(Calcaterra et al., 2010)* RNA binding protein that regulates translation of various proteins, including ornithine decarboxylase (ODC), the rate limiting enzyme of polyamine metabolism (*Coni S. 2019; Casero et al., 2018; Wallace, 2000).* Microsatellite expansions of CCUG repeats in the first intron of the *CNBP* gene are associated to type 2 myotonic dystrophy (DM2) *(Liquori et al., 2001; Sammons et al., 2011; Wallace et al., 2003).* However, whether the clinical manifestations of DM2 are related to CNBP and polyamine metabolism is not known. To investigate the role of CNBP in muscular diseases we performed *in vivo* studies using *Drosophila Melanogaster* as a model organism. We show that depletion of dCNBP in fly muscles, obtained by using different drivers, specific for different muscle tissues, causes locomotor defects that are linked to an impaired polyamine metabolism. We demonstrate that, upon dCNBP depletion, the levels of ornithine decarboxylase (ODC) and polyamines are significantly reduced, and that ODC silencing phenocopies the dCNBP-dependent locomotor defects. We demonstrate that dCNBP controls polyamine metabolism by binding dODC mRNA and regulating its translation. Moreover, the dCNBP-dependent locomotor defects are rescued by either polyamine supplementation or dODC1 overexpression. Finally, we show a strong correlation between CNBP and polyamines levels in muscle cells from DM2 patients, which are both downregulated compared to heathy individuals. Collectively our results revealed an unprecedented role of CNBP in muscles diseases, conserved between *Drosophila* and Humans which consist in the regulation of polyamine metabolism and consequently muscle function. The CNBP-ODC-Polyamines axis unveil in this context could represent a druggable target in muscle diseases like the DM2.

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[L10]

## Dysregulation of the polyamine system in favor of its catabolism is a common mechanism after kidney injury

T. Sieckmann[1], N. Ögel[1], S. Kelterborn[1], F. Boivin[2 4], G. Schley[5], M. Fähling[1], M. I. Ashraf[3], M. Reichel[2], E. Vigolo[4], A. Hartner[6], F. Knauf[2], C. Rosenberger[2], F. Aigner[3, 7], K. Schmidt-Ott[2 4], H. Scholz[1], K. M. Kirschner[1]

[1] *Institute of translational Physiology,*

[2] *Department of Nephrology and Medical Intensive Care,*

[3] *Department of Surgery, Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany*

[4] *Molecular and Translational Kidney Research, Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany*

[5] *Department of Nephrology and Hypertension, Friedrich-Alexander University Erlangen-Nürnberg (FAU) and University Hospital Erlangen, Erlangen, Germany*

[6] *Department of Pediatrics and Adolescent Medicine, Friedrich-Alexander University Erlangen-Nürnberg (FAU) and University Hospital Erlangen, Erlangen, Germany*

[7] *Department of Surgery, St. John of God Hospital Graz, Graz, Austria*

*tobias.sieckmann@charite.de*

It is known that certain genes of the polyamine system are dysregulated after kidney ischemia reperfusion injury in mice. We hypothesise that different forms of acute and chronic kidney injury lead to similar changes in the expression patterns of the polyamine system. In eight different murine models of acute and chronic kidney injury expression of genes involved in polyamine homeostasis was analyzed by RT-qPCR and RNAScope. Interestingly, the expression of catabolic enzymes (*Aoc1* and *Sat1*) was upregulated, while the anabolic enzymes (*Odc1*, *Sms*) were downregulated. The same trend was observed in a RNA sequencing data sets (NCBI Gene Expression Omnibus) of transplanted donor kidneys in humans. The putrescine-degrading enzyme AOC1 exhibits the most striking changes. The detected increase of *Aoc1* can be located to injured but regenerating proximal tubules. By overexpression of AOC1 in the human embryonic kidney (HEK293) cell line we could show that AOC1 can lead to elevated autophagy activity. As a screening for stimuli of increased *Aoc1* expression, we used mouse embryonic kidney explants. Here we observed changes of *Aoc1* expression under hypoxia and hyperosmotic conditions. These changes were further examined in mouse models of hypoxia. However, in vivo, hypoxia did not lead to changes of Aoc1 expression. Hyperosmolarity was confirmed as a dose-dependent stimulus of *Aoc1* by using the kidney cell lines M15 and 209/MDCT as well as cultured primary proximal tubules. Using reporter gene and RNA-stability assays, we could show that the increase in *Aoc1* expression is based on mRNA-stabilization and transcriptional activation. siRNA knockdown experiments in M15 cells showed that transcriptional activation of *Aoc1* in hyperosmolar conditions is mediated by the transcription factor NFAT5. Notably, murine expression of *Aoc1* is regulated by three different promoters. One of the promoters driving expression of the *Aoc1* splice isoform 205 (Ensemble database) was strongly stimulated by hyperosmolar conditions as shown in luciferase promoter assays. The *Aoc1* isoform 205 is also the one most strongly upregulated after kidney ischemia reperfusion. We could show that the AOC1 splice variant 205 contains an additional set of 22 amino acids N-terminally that lead to an altered subcellular localization and increased secretion. In conclusion, different models of kidney injury exhibit a similar pattern of dysregulation of the polyamine system with the most striking change being the upregulation of *Aoc1* in proximal tubules. Using hyperosmolarity as a stimulus, we established a model to study polyamine function and provide first insights into the regulation of *Aoc1* under damaging conditions.

## SESSION 4

*Polyamines in human health:*
*in cancer and other diseases. Therapeutic applications*

[PL11]

## Targeting the Immunomodulatory Effects of Polyamines in Cancer

Eric Alexander[1], Sharon Shania[1], Olivia El Naggar[1], Erin Fahey[1], Otto Phanstiel[2], Susan Gilmour[1]

[1] *Lankenau Institute for Medical Research, Wynnewood, PA*

[2] *University of Central Florida, Orlando, FL*

*GilmourS@MLHS.org*

To starve tumors of polyamines that are essential for their growth and survival, we have developed a polyamine-blocking therapy (PBT) that includes *a combination of* α-difluoromethylornithine (DFMO) and a novel polyamine transport inhibitor (PTI), i.e., a three-armed-polyamine compound (Trimer PTI). Previous studies have demonstrated that the anti-tumor effect of PBT in a variety of animal tumor models is T-cell dependent and is accompanied by increased tumor infiltration and activation of tumor-specific $CD8^+$ T-cells and a decrease in immunosuppressive tumor infiltrating cells including myeloid derived suppressor cells (MDSCs) and M2-like macrophages. PBT treatment reduces cyto-protective autophagy in tumor-infiltrating MDSC and macrophage subpopulations, blunts M2-like alternative activation of macrophages while increasing the differentiation of $CD80^+$, $CD11c^+$ macrophages, and reduces STAT3 activation in MDSC cultures. PBT significantly enhances the anti-tumor efficacy of PD-1 blockade in both 4T1 and B16F10 tumors that are resistant to anti-PD-1 monotherapy, increasing tumor-specific cytotoxic T-cells and survival of tumor-bearing animals beyond that in mice treated with PBT or PD-1 blockade alone. Our results highlight the powerful activity of PBT treatment in re-invigorating the T-cell directed activity of immune checkpoint blockade and promoting immune-mediated elimination of tumor cells. In addition, we have investigated another novel polyamine transport system-targeted, cytotoxic, arylmethyl-polyamine (*AP*) compound in melanoma models. $BRAF^{V600E}$ mutant melanoma cells are more sensitive to *AP* that exploits their increased polyamine uptake compared to that of BRAF wildtype cells. Using an animal model of BRAF inhibitor-resistant melanoma, co-treatment with the BRAF inhibitor, PLX4720, and *AP* significantly delays the recurrence of PLX4720-resistant melanoma tumors and decreases tumor-promoting macrophages. Development of BRAF inhibitor-resistance enriches for metastatic cancer stem cells (CSC) and increases tumor-promoting macrophages. *In vitro* studies demonstrated that $CD304^+$, $CXCR4^+$ spheroid cultures of BRAF mutant melanoma cells are resistant to PLX4720 but are more sensitive to *AP* compared to monolayer cultures of the same cells. *AP* significantly inhibits melanoma cell invasiveness across a Matrigel-coated filter using the CXCR4 ligand, SDF-1α, as the chemoattractant, blocks SDF-1α-chemotaxis of $CXCR4^+$ macrophages, and also inhibits M2 polarization of macrophages. In melanoma-macrophage co-cultures, *AP* prevents the PLX4720-induced release of pro-tumorigenic growth factors, such as VEGF, from macrophages and prevents the macrophage rescue of BRAF mutant melanoma cells treated with PLX4720. *AP* offers exciting potential as an adjunct cancer treatment to treat chemo-resistant melanoma because it targets the polyamine transport system in BRAF inhibitor-resistant CSCs and also blocks CXCR4 signaling in invasive melanoma cells and pro-tumorigenic macrophages. (Supported by DOD grant CA150356)

[L12]

## ATP13A3 in polyamine homeostasis and in the pathogenesis of Pulmonary Arterial Hypertension

Mujahid Azfar, Nathalie Jacobs, Jialin Chen, Bin Liu, Norin Hamouda, Shaun Martin, Sarah Van Veen, Nicholas Morrell, Peter Vangheluwe

*mujahid.azfar@kuleuven.be*

Intro: We recently established the P5B ATPase ATP13A2 as a novel mammalian polyamine (PA) transporter. Consequently, other P5B ATPases are now being studied as candidates of the elusive mammalian PA transport system as they all share a conserved substrate binding site, predicted-structural homology and reside in overlapping endo/lysosomal compartments. In this context, we study ATP13A3 and its role in overall PA homeostasis. ATP13A3 has been implicated in several pathologies marked by a dysregulated PA homeostasis. However, its strongest implication is in a rare cardiovascular disease called Pulmonary Arterial Hypertension (PAH), where whole genome sequencing in PAH patients revealed several heterozygous point, frame-shift or truncation mutations. Methods: We used lentiviral transduction in human microvascular endothelial (HMEC-1) cells and human neuroblastoma (SH-SY5Y) cells to generate stable cell lines to either knock-down (KD) ATP13A3, or over-express (O.E.) the wild-type (Wt), catalytically dead (DN), or PAH disease mutants of ATP13A3. We then measured the effect of modulating ATP13A3 expression on cellular PA uptake using fluorescentlytagged PAs, endogenous PA levels via metabolomics and PA toxicity. We also characterised the PAH mutants to assess their potential role in the disease pathogenesis. Moreover, using Saccharomyces cerevisiae as an expression host, we purified hATP13A3 to perform dedicated biochemical assays on the isolated protein. Results: ATP13A3 Wt O.E., but not DN O.E., sensitises cells to increasing PA concentrations suggesting a role in PA uptake, which was confirmed with uptake assays using fluorescentlytagged PAs. PA uptake was attenuated using a PA transport inhibitor benzyl viologen as well as by ATP13A3 KD. ATP13A3 KD also reduced the overall intracellular PA pool. We also demonstrate that the heterozygous ATP13A3 missense mutations in PAH patients present a loss-of-function phenotype unable to promote cellular PA uptake. Conclusion: We demonstrate that ATP13A3 is a member of the mammalian polyamine transport system that regulates PA uptake and homeostasis, whereas the identified PAH mutations impair ATP13A3 activity solidifying that ATP13A3 dysfunction is implicated in PAH pathogenesis. The purification of ATP13A3 protein is a major step forward to conclusively establish ATP13A3 as a PA transporter via the use of biochemical assays.

[L13]

## Polyamine metabolism in the pathology and treatment of Snyder-Robinson syndrome

Tracy Murray Stewart, Jackson R. Foley, Robert A. Casero, Jr.
*Johns Hopkins University School of Medicine, Baltimore, MD, USA*
*tmurray2@jhmi.edu*

Snyder-Robinson syndrome (SRS) results from mutations in the spermine synthase (*SMS*) gene, resulting in reduced or lost function and decreased or eliminated conversion of spermidine into spermine (1). The X-linked SRS phenotype is characterized by osteoporosis, hypotonia, seizures, and intellectual disability, with variations in severity that tend to correlate with SMS activity level (2). Existing treatments target symptoms, not underlying causes. SRS patient cells accumulate excess spermidine, while spermine levels are reduced, relative to wildtype cells; the extent to which this ratio is elevated depends on the specific *SMS* mutation. Although dietary spermine does not appear to benefit SRS patients or mouse models, we have shown that SRS cells maintain functional polyamine transport and homeostatic control systems that respond to exogenous spermine and growth-supporting spermine mimetics by reducing spermidine to within normal levels (3,4). This knowledge led to our current studies investigating ways in which to exploit polyamine homeostatic control mechanisms in support of a more normal polyamine profile. As most SRS-causing mutations only partially reduce SMS enzymatic activity, improving the conversion of spermidine into spermine in hypomorphic *SMS* cells is a focus of our studies. Using established modulators of polyamine metabolism, we clarify the mechanisms through which inhibition of polyamine biosynthesis can stimulate this conversion in SRS-affected cells, providing important insight suggesting additional therapeutic approaches to *reduce the aberrant spermidine/spermine ratio in SRS cells. These include combination strategies to simultaneously increase polyamine transport, reduce spermidine biosynthesis, and enhance spermine biosynthesis, thereby facilitating the re-establishment of polyamine homeostasis and forming a foundation for future translational studies with significant therapeutic potential.*

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[PL14]

## DFMO Treatment of Children with ODC1-linked Bachmann-Bupp Syndrome: From Discovery to Clinic

Andre S. Bachmann*[1], Chad R. Schultz[1], Elizabeth A. Vansickle[2], Julianne Michael[2], Jeremy W. Prokop[1], Surender Rajasekaran[2], Caleb P. Bupp[2]

[1] *Michigan State University, Department of Pediatrics and Human Development, Grand Rapids, United States of America*

[2] *Spectrum Health and Helen DeVos Children's Hospital, Grand Rapids, United States of America*

[*] *Presenting Author*

Bachmann-Bupp Syndrome (BABS) is an autosomal dominant genetic disorder caused by heterozygous *de novo* variants in the ornithine decarboxylase 1 (*ODC1*) gene (OMIM #619075). ODC is a rate-limiting enzyme in the polyamine pathway that plays a key role in physiological and developmental processes during embryogenesis, organogenesis, and neoplastic cell growth. The first BABS patient was seen at Helen DeVos Children's Hospital and diagnosed by whole exome sequencing (WES). Red blood cells (RBCs) and primary skin fibroblasts were analyzed for ODC protein levels, enzymatic activity, and polyamines (putrescine, spermidine, spermine) using western blot, 14-C radioactive ODC assay, and RP-HPLC, respectively. Computational modeling was performed to depict a 3D structure of ODC and its truncated variants. Under an FDA-approved single patient IND, the patient has been treated with oral ODC inhibitor DFMO (eflornithine) for 32 months (ongoing) and serial blood samples analyzed by LC-MS/MS to monitor the patient's metabolome. WES revealed a heterozygous *de novo* nonsense mutation in the *ODC1* gene that leads to a premature abrogation of 14-aa residues at the ODC protein C-terminus. Phenotypic manifestations included macrosomia, macrocephaly, developmental delay, alopecia, spasticity, hypotonia, cutaneous vascular malformation, delayed visual maturation, and sensorineural hearing loss. RBCs and primary dermal fibroblasts showed elevated levels of ODC protein, ODC enzyme activity, and putrescine levels compared to healthy controls. Treatment of BABS patient with DFMO reduced N1-acetylputrescine in blood and led to significant clinical improvements. A total of 12 BABS patients are now known worldwide. These patients benefit from treatment with a repurposed, FDA-approved drug, DFMO/eflornithine.

[L15]

## Spermine metabolite, hydrogen peroxide and aldehyde, cause apoptosis in neuroblastoma cells associated with increase of p53, Caspase-3 and miR-34a

Enzo Agostinelli

*Department of Sensory Organs, University of Rome "La Sapienza", Policlinico Umberto I, Viale del Policlinico 155, 00161, Rome, Italy and International Polyamines Foundation 'ETS-ONLUS', Via del Forte Tiburtino 98, 00159 Rome, Italy.*

*E-mail: enzo.agostinelli@uniroma1.it*

Neuroblastoma (NB) is a common malignant solid tumor in children, which originates from the sympathoadrenal lineage of neural crest and accounts for 15% of childhood cancer mortality. Amplification of the oncogene N-Myc is a well-established poor prognostic marker for neuroblastoma. Whilst N-Myc amplification status strongly correlates with higher tumour aggression and resistance to treatment. Therefore, new therapies for patients with N-Myc amplified NB need to be developed. The *in situ* formation of cytotoxic polyamine metabolites by bovine serum amino oxidase (BSAO) is a recent approach in cancer enzymotherapy. It was demonstrated that BSAO and spermine (SPM) addition to cancer cells induces cell growth inhibition and apoptosis through the oxidative stress caused by polyamine metabolites, $H_2O_2$ and aldehydes, produced by the oxidative reaction [1]. The cytotoxic effect induced by BSAO and SPM was evalulated by both a clonogenic and MTT assays. The detection of apoptosis in NB cells was evaluated by flow cytometry after Annexin V-FITC labelling and DNA staining with propidium iodide. The percentages of Annexin V-positive cells matched quite well with that of cells showing hypodiploid sub-G1 peak. An increase in mitochondrial membrane depolarization (MMD) was found in NB cells treated with the enzymatic system. The mitochondrial membrane potential activity was checked by flow cytometry studies, labelling cells with the probe JC1 dye. We also analysed by real time RT-PCR the transcript of some genes involved in the apoptotic process, to determine possible down or up regulation of mRNAs after the treatment of the SJ-N-KP and the N-Myc amplified IMR-5 cell lines with BSAO and SPM. [2]. The experiments were carried out considering the pro-apoptotic genes P53, PUMA and CASPASE-3. After treatment with BSAO and SPM, both cell lines displayed increased mRNA levels for all these pro-apoptotic genes. Interestingly, the pro-apoptotic Sirt-1 inhibitor microRNA miR-34a significantly increases in SJ-N-KP and IMR5 cells treated with BSAO and SPM. Western blotting analysis with PARP and Caspase 3 antibody support the concept that BSAO/SPM treatment induces high levels of apoptosis in NB cell lines [2]. The major conclusion is that BSAO/SPM treatment leads to anti-proliferative and cytotoxic activity of both NB cell lines, associated with activation of apoptosis. Moreover, the findings suggested that enzymatic spermine metabolite could be a powerful tool in the development of new anticancer treatments.

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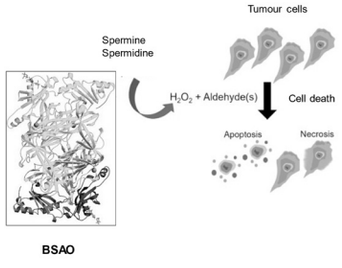

[L16]

## Polyamine blockade inhibits cell growth and induces apoptosis in high-risk childhood leukaemia

Weiman Gao[1 2*], Mawar Karsa[1 2*], Lin Xiao[1 2], Ruby Pandher[1 2], Emma Ronca[1], Angelika Bongers[1], Murray D. Norris[1 2], Michelle Haber[1 2], Klaartje Somers[1 2]

[1] *Children's Cancer Institute, Lowy Cancer Research Centre, UNSW Sydney, Sydney, NSW, Australia.*

[2] *School of Women's and Children's Health, UNSW Sydney, Sydney, NSW, Australia.*

[*] *These authors contributed to this work equally.*

*Email address of presenting author: lgao@ccia.org.au*

**Introduction**: Leukaemia is the most common cancer in children. While the survival rates of childhood leukaemia are approaching 90%, the prognosis of high-risk leukaemia subtypes including treatment-refractory ALL, T-ALL and ALL with rearrangements of the *MLL/KMT2A* gene, remains extremely poor. In addition, leukaemia survivors often endure long-term treatment side effects, negatively impacting quality of life. Therefore, it is critical to develop better and less toxic treatment approaches for high-risk childhood leukaemia. Cancer cells are often addicted to polyamines and frequently upregulate polyamine synthesis to fuel their rapid growth, which has resulted in the development of several drugs that selectively target cancer cells by inhibiting polyamine pathways. DFMO, an inhibitor of the rate-limiting polyamine synthesis enzyme, ODC1, is currently under investigation as a potential therapy for solid cancers. However, the effects of targeting the polyamine pathway in leukaemia are understudied.

**Objective**: To determine the preclinical efficacy of polyamine inhibition therapy in high-risk paediatric leukaemia.

**Methods and Results**: In the Target II paediatric B-ALL cohort (n=203), high ODC1 expression is associated with shorter event-free survival, suggesting a role for the polyamine pathway in leukaemia progression. Although DFMO reduced leukaemia cell viability in a dose-dependent manner, we observed that upon treatment with DFMO, leukaemia cells induced compensatory increases in polyamine uptake from the extracellular environment. Addition of AMXT1501, a polyamine mimetic that blocks polyamine uptake, abolishes the increased polyamine uptake induced by DFMO and reduced intracellular polyamine levels. The combination of DFMO and AMXT1501 synergistically decreased the viability of a diverse panel of leukaemia cell lines, including cells derived from treatment-refractory patients, through inducing apoptosis. We are currently testing the combination of DFMO and AMXT1501 in patient-derived xenograft models of high-risk leukaemia and are determining markers of response.

**Conclusions**: Polyamine depletion by DFMO and AMXT1501 constitutes a promising therapeutic strategy for treatment of high-risk leukaemia.

## SESSION 5

*Polyamines in nutrition and longevity*

[L17]

## Spermidine treatment affects gene expression in mouse model of Amyotrophic Lateral Sclerosis

C. Fiorucci[1], M. N. Rossi[1], O. Carletta[1 2], S. Giuliani[1], M. Terricola[1], S. Scaricamazza[3], F. Berardinelli[1 2], A. Ferri[3 4], C. Valle[3 4], Moreno S.[1 3], P. Mariottini[1], Manuela Cervelli[1 3]

[1] *Department of Science, University Roma Tre, Rome, Italy.*

[2] *Division of Experimental Neuroscience, IRCCS Fondazione Santa Lucia, Rome, Italy*

[3] *Laboratory of Neurodevelopmental Biology, Neurogenetics and Molecular Neurobiology, IRCCS Fondazione S. Lucia, Rome, Italy.*

[4] *National Research Council, Institute of Translational Pharmacology (IFT), Rome, Italy.*

*Email of presenting author: manuela.cervelli@uniroma3.it*

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of the upper and lower motor neurons. Alterations in lipid metabolism and oxidative stress, related to mitochondrial dysfunction have been described in ALS patients and play crucial roles in disease onset and progression. In spite of the growing effort in understanding the etiopathology of ALS no therapies are currently available to block its progression (Candelise et al., 2022). Spermidine supplementation exerts neuroprotective effects in different *in vivo* neurodegenerative models. In *Drosophila* spermidine protects from age-induced memory impairment and loss of locomotor activity in an autophagy-dependent manner. In a mouse model of multiple sclerosis, oral supplementation of spermidine attenuates disease progression and improves visual functions through the reduced demyelination of optic nerve and spinal cord and decreased loss of retinal ganglion cells (Madeo et al., 2018). On the base of the neuroprotective effects of spermidine we decide to treat SOD1G93A mouse model of ALS at the onset of the symptoms. Spermidine was administrated for 50 days and then gene expression was analysed from spinal cord and gastrocnemius to evaluate neurodegeneration and muscle atrophy, respectively. Our analysis, that includes transcription factors as well as their target genes involved in the energetic metabolism, revealed that spermidine administration is able to counteract the molecular changes occurring in spinal cord and muscle of ALS mouse model. In conclusion, our data suggest a positive effect of spermidine on mouse phenotype opening the way for future studies aimed at improving patients' outcome.

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[L18]

## Aging associated change in polyamine metabolism

Takeshi Uemura[1 2], Yuka Yokota[1], Miki Matsunaga[1], Yoshihisa Akasaka[2], Koichi Takao[1], Hiroshi Ikegaya[2], Takemitsu Furuchi[1]

[1] *Faculty of Pharmacy and Pharmaceutical Sciences, Josai University*

[2] *Department of Forensic Medicine, Kyoto Prefectural University of Medicine*

*e-mail: t_uemura@josai.ac.jp*

Polyamines are bioactive amines present in almost all living organisms and are essential for normal cellular functions. Cellular polyamine levels are regulated by biosynthesis, degradation, and transport. Recently, it has been suggested that age-related changes in polyamine levels are associated with various age-related diseases such as cerebral infarction (*1*). We found that oxidative degradation of spermine is elevated in brain tissue of aging mice and that the degradation byproduct acrolein is strongly associated with increased risk of cerebral infarction (*2*). Acrolein is a highly reactive unsaturated aldehyde that binds to proteins and impairs their function (*3, 4*). Elucidating the mechanisms of age-related changes in polyamine metabolism will enable the development of preventive methods for age-related diseases. In this study, we investigated the relationship between aging and tissue polyamine levels and polyamine metabolism using HepG2 cells and human liver tissue obtained by autopsy. Polyamine analysis of liver tissue revealed a decrease in spermine and an increase in putrescine and protein-bound acrolein content with increasing age. Polyamine metabolic enzyme levels were measured and found that spermine oxidase (SMOX), which catalyzes the oxidative degradation of spermine, increased with age. On the other hand, ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AMD1), the rate-limiting enzymes for polyamine biosynthesis, decreased with age. It was found that the amounts of putrescine, spermidine, spermine, and protein-bound acrolein in liver tissue showed a significant correlation with the age of subjects (*5*). These results suggest that an increase in polyamine degradation and a decrease in biosynthesis with aging play an important role in changes in tissue polyamine levels. We next tested the effect of polyamine catabolism inhibitor on cell aging using HepG2 dells. MDL72527, an inhibitor of polyamine degradation, suppressed a senescence associated increase in b-galactosidase activity. Our results suggest that age-related diseases can be prevented by reducing the increase in polyamine degradation and decrease in biosynthesis due to aging, maintaining tissue polyamine balance, and suppressing acrolein production.

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2., T. Uemura *et al.*, Protective Effects of Brain Infarction by N-Acetylcysteine Derivatives. *Stroke* 49, 1727-1733 (2018).

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[L19]

## Nutritional value of Spermidine for *Strombidium sp.* NTOU1, a marine ciliates, and its potential impact on the ocean food chain and ecosystems

Bo-Ying Su[1], Hung-Yun Lin[1 2], Han-Jia Lin[1 2]

[1] *Department of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan*

[2] *Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan*

Polyamines exist in almost all living organisms and are essential for life-sustaining. The role of dietary polyamines in mammals has been extensively studied, but research on their effects on zooplankton is still rare. *Strombidium* sp. NTOU1, a marine ciliate, is one of the main predators in the microbial ecosystem. In the past, it has also been used as a biological control species for bacterial diseases, or as a biological feed for aquatic seedlings. In the establishment of a large-scale cultivation technology of ciliates, it was found that by using *E. coli* which can produce a large amount of Spd after genetic modification, the growth rate of ciliates is 1.4 times faster than that feeding with normal *E. coli*. On the other hand, adding Spd directly to the environment cannot increase the growth rate of ciliates.

In order to study the effects of different types of polyamines on the growth of ciliates, We first added different types of polyamines to the *E. coli* culture medium. The results found that Put, Cad, Dap, NSpd, NSpm, etc. can all be absorbed by *E. coli.* and intensively accumulate in cells. Then, these *E. coli*, which are rich in various polyamines, were used as bait to feed the ciliates, and it was found that the polyamine composition in the ciliates was indeed changed. However, with the exception of Spd and NSpd, most polyamines have no significant effect on the growth rate of ciliates. Feeding *E. coli* containing NSpd would cause the growth rate of ciliates to decrease.

Other physiological effects of *Strombidium* sp. NTOU1 induced by Spd accumulation were also tested, including ciliate predation capacity, antioxidant capacity, and resistance to osmotic stress. The results show that the intracellular accumulation of Spd in *Strombidium* sp. NTOU1 can promote its feeding speed, which may be one of the factors that Spd promotes cell growth. Under osmotic and oxidative stress, the growth inhibition of *Strombidium* sp. NTOU1 could be rescued by feeding Spd-rich diets. These results suggest that Spd may be related to the mechanism by which *Strombidium* sp. NTOU1 adapts to osmotic pressure and oxidative stress.

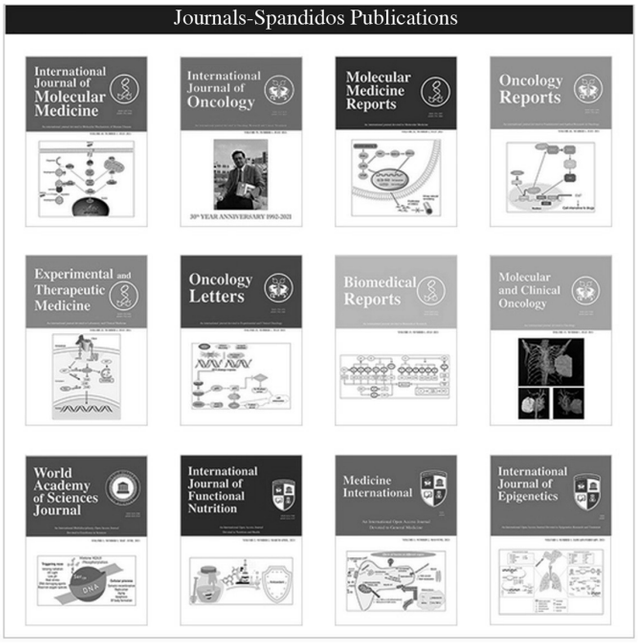
[L20]

# Publishing in Biomedical Sciences

Demetrios A. Spandidos

*Medical, School, University of Crete, Heraklion, Greece 2Spandidos Publications, Athens, Greece and London, UK Spandidos*

Publications was founded in 1992 and has developed into a leading publishing group in the biomedical sciences field. We currently publish twelve journals: International Journal of Molecular Medicine, International Journal of Oncology, Molecular Medicine Reports, Oncology Reports, Experimental and Therapeutic Medicine, Oncology Letters, Biomedical Reports, Molecular and Clinical Oncology, World Academy of Sciences Journal, International Journal of Functional Nutrition, Medicine International and International Journal of Epigenetics. When deciding which journal to submit your paper, you should consider who would be interested in your results. A small number of scientists working in your special field only, or would it be of interest to others working in complementary or parallel fields. Next, evaluate the level of significance of your findings. In this process your colleagues will be of great help, as very few of us are critical enough of our own work. Overestimation, might cost you many months of frustration and uncertainty. Consider carefully whether there is a need to submit to a so-called prestigious journal, where the disappointment is very likely. Any established journal will forward the Abstracts of all published works to various databases, thus yours will have equal opportunity to be noticed, regardless of the journal in which it is printed. It is of interest to study how long the time period is from publishing to finding the entry in, for example Medline or Science Citation Index; as these are the 'advertising windows' frequently used in search of new information. This leads us to the next very important point to establish how long the review and publications process is likely to be. Previous issues will indicate the trend, and this is of major importance, as the process varies greatly from a few weeks to over one year. Thus, before submitting do some research on rapid publication, if you need a reply within a reasonable time period. A strategy for a successful publication shall be considered



## SESSION 6

*Polyamines and their analogs: chemistry and molecular pharmacology*

[PL21]

## The effect of subtituted amino-alkyl-anthraquinones on eukaryotic cells

Uriel Bachrach

*Department of Molecular Biology, Hebrew University-Hadassah Medical School, Jerusalem, Israel*

*urielb@ekmd.huji.ac.il*

Anthraquinone is a polycyclic aromatic hydrocarbon derived from anthracene or phthalic anhydride. Anthraquinone) , is used in the manufacture of dyes and it also exhibits some anti-bacterial and anti-cancer activities. However, at higher doses , (250 mg/kg weight) it can cause damage to mouse DNA and induce mutations in bacteria (2 µg /plate). To overcome these difficulties, we decided to synthesize aminoalkyl anthraquinone derivatives which bind to cellular nucleic acids and affect their activities. The chemists (from the School of Pharmacy) synthesized 52 derivatives of anthraquinones, having polymine side chains at differernt sites of the polycyclic aromatic hydrocarbons. These, positively charged alkyl derivatives, bind to cellular nucleic acids and impair their activities. We studied: 1. Anti-Cancer activity (5 tumor cells), 2. Anti-bacterial activity, 3. Anti-viral activity (bacteriophages), 4. Tropical Medicine (malaria and leishmaniasis ( 4 different species ) ), 5 . Inhibition of the activity of bovine serum amine oxidase. 6 . Testing the toxicity by injecting into growing chick embryos. The aminoalkyl-anthraquinones ( at the concentrations of 4- 170 µ M ) , inhibited the growth of all the cells tested . The effect was most significant when parasites causing Tropical Diseases were examined. To test the toxicity of the aminoalkyl-anthraquinones**,** solutions ( 0.2 ml) containing 0.07-3.57 µmoles of the drugs, were injected into the allantoic fluid of 6 chick embryo of 12 days old eggs . After incubation, (a) viability was assessed by checking movement of the body, eyes and heart, (b) developmental defects of eyes, wings and feathers were examined , and (c) the embryo was weighed. Compounds V and IV did not change the average weight of the embryos and did not affect their development. This is a very sensitive method to test toxicity. Obviously, the compounds tested lacked toxicity.

### Conclusions

This study strongly suggests that aminoalkyl-anthraquinones play a promising scaffold for the development of therapeutic agents. Polyamines and diamines are practically present in all living cell. There they control growth and differentiation processes Aminoalkyl-anthraquinones can compete with these organic cations and block their activities.

[L22]

## Mechanism of action for an alkylated polyamine analogue diethylnorspermine (DENSPM) in treating pheochromocytoma/paraganglioma

Sergei G Tevosian[1], Abdel A. Alli[2], Niharika Bala[2], Raymond Bergeron[3], Hans K Ghayee[4 5], Heather M. Hatch[1], Yiling Xu[4]

[1] *Department of Physiological Sciences, University of Florida, Gainesville FL*

[2] *Department of Physiology and Functional Genomics, College of Medicine Gainesville FL*

[3] *Department of Medicinal Chemistry, University of Florida, Gainesville FL*

[4] *Division of Endocrinology & Metabolism, University of Florida*

[5] *Malcom Randall VAMC, Gainesville FL, USA*

Pheochromocytomas (PCC or PHEO) and paragangliomas (PGL) are tumors of the neuroendocrine system. To successfully treat this disease, it is important to identify suitable biomarkers to predict plausible clinical benefits, endpoints and success of combination treatments. We have previously established progenitor cells (hPheo1) from a patient's PCC tumor and produced its derivative, an *SDHB* knockdown line. Metabolomics analysis suggested that polyamine pathway is highly active in PCC/PGL tumors and cells with SDHB deficiency. Utilizing a synthetic polyamine analogue $N^1$, $N^{11}$-diethylnorspermine (DENSPM), we further discovered that DENSPM is effective in suppressing the growth of wild-type parental hPheo1 and *SDHB* mutant hPheo1 cells in culture, and in animals carrying xenograft tumors derived from these cells. To delineate the mechanism of DENSPM action in PCC, we now performed an RNAseq analysis using DENSPM-treated hPheo1 cell line in parallel with its CRISPR-Cas SDHB-deficient derivatives and identified several informative candidates. To confirm RNAseq-derived datasets, we completed real-time PCR and Western blotting analysis of untreated and DENSPM-treated cells. We also show that animal xenografts derived from these cells undergo a similar change in gene expression pattern upon polyamine inhibitor treatment. Our results now unequivocally demonstrate that polyamine inhibitors suppress two key biochemical pathways critical for rapidly dividing cancer cells: lipid/fatty acid metabolism based on desaturase enzyme SCD1 and mitochondrial stress response. Moreover, we also identified genes differentially expressed in parental and DSDHB mutant hPheo1 cells. Compared to control non-targeting siRNAs, siRNA-mediated knockdown of SCD1 in hPheo1 wild-type and SDHB knockdown cells resulted in notable changes in their lipid composition. Our findings are significant since cancers with DSDHB mutation are associated with metastatic and uncurable disease in patients. These data point to a previously unrecognized mechanism of polyamine pathway action in *SDHB* mutated related cell culture models.

[L23]

# Ligand-Based and Structure-Based Studies on Polyamine Analogues as Bovine Serum Amine Oxidase Substrates

Rino Ragno[1], Eleonora Proia[1], Antonini Lorenzo[1], Lavinia Rutigliano[2], Alessandro Montella[2], Tomoaki Tahara[2], Giancarlo Altissimi[2], Alessio Ragno[3 4], Roberto Capobianco[3 4], Gianluca Canettieri[5 6 7], Enzo Agostinelli[2 7]

[1]*Rome Center for Molecular Design, Department of Drug Chemistry and Technology, Sapienza Università di Roma, P. le A. Moro 5, 00185 Roma, Italy*

[2]*Department of Sensory Organs, Sapienza University of Rome, Policlinico Umberto I, Viale del Policlinico 155, 00161 Rome, Italy*

[3]*Dept. of Computer, Control and Management Engineering "Antonio Ruberti", Sapienza University of Rome*

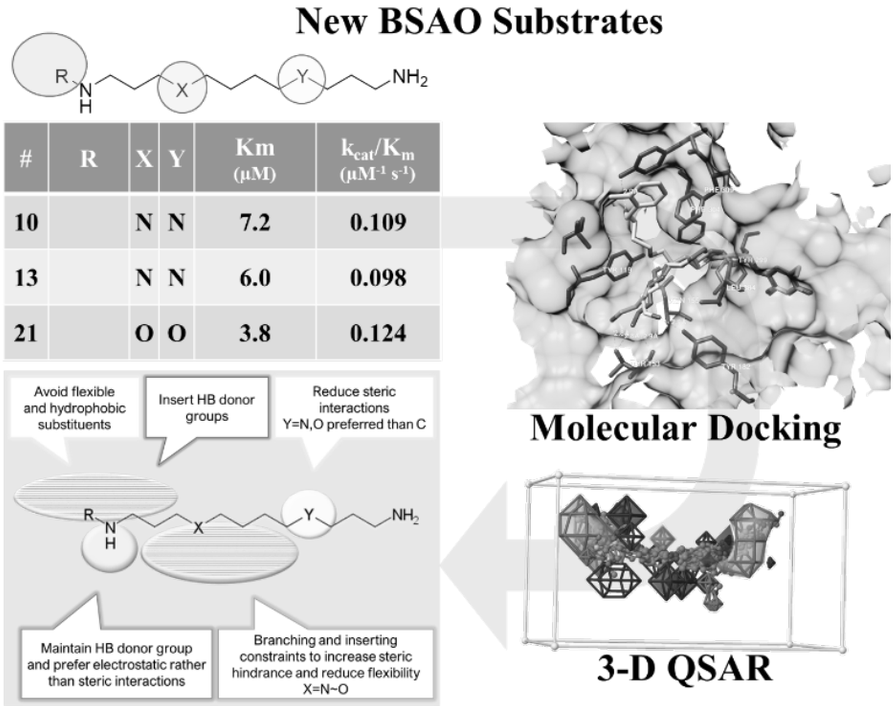
[4]*Sony AI*

[5]*Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena 291, 00161 Rome, Italy and* [6]*Istituto Pasteur, Fondazione Cenci-Bolognetti, Sapienza University of Rome, Viale Regina Elena 291, 00161 Rome, Italy*

[7]*International Polyamines Foundation 'ETS-ONLUS' Via del Forte Tiburtino 98, I-00159 Rome, Italy*

Abstract

Natural polyamines (PAs) are key players in cellular homeostasis by regulating cell growth and proliferation. Several observations highlight that polyamines (PAs) are also implicated in pathways regulating cell death. Indeed, the PAs accumulation cytotoxic effect, maximized with the use of Bovine Serum Amine Oxidase (BSAO) enzyme, represents a valuable strategy against tumor progression. In the present report, a mixed Structure-Based (SB) and Ligand-Based (LB) protocol was applied on a list of PAs as BSAO substrates. Binding modes of BSAO-PA modeled complexes led to clarify electrostatic and sterical features likely affecting the BSAO-PA biochemical kinetics. LB and SB Three-Dimensional Quantitative Structure-Activity Relationships (Py-CoMFA and Py-ComBinE) models were developed by means of the 3d-qsar.com portal and their analysis represent a strong basis for future design and synthesis of PAs BSAO substrates for potential application in oxidative stress-induced chemotherapy.



[L24]

## Development of FUBP1 inhibitors to control cancer cell growth

Holly Moots, Aiste Dobrovolskaite, Mukund Tantak, Alex Bunea, Christopher Polera, Chloe Robinson, Otto Phanstiel

*12722 Research Parkway, Department of Medical Education, University of Central Florida College of Medicine, Orlando, Florida, USA 32826.*

*Otto.Phanstiel@ucf.edu*

**Abstract:** The far upstream binding protein 1 (FUBP1) regulates the transcription of several tumor survival genes by binding to their specific far upstream element (FUSE) DNA sequence. Disrupting this specific DNA-protein interaction provides for a novel way to control the expression of specific FUSE-controlled genes including c-Myc. Ornithine decarboxylase is a rate limiting enzyme of polyamine biosynthesis and c-Myc is a known ODC transcriptional activator. Since c-Myc is regulated by FUBP1, FUBP1 inhibitors are expected to inhibit c-Myc expression and affect downstream polyamine homeostasis. Here, we report the discovery and development of novel FUBP1 inhibitors and demonstrate their ability to inhibit c-Myc transcription, reduce c-Myc protein levels and decrease polyamine levels in human pancreatic cancer cells. Interestingly, the tumor suppressor p21 is a FUSE controlled gene which is negatively regulated by FUBP1. In the presence of our FUBP1 inhibitor, both p21 transcription and p21 protein increase. In sum, the ability to control multiple FUSE directed genes (c-Myc and p21) with a single molecule provide a novel upstream approach to inhibit polyamine biosynthesis and control cell growth.

[L25]

## Characterization of novel green fluorescent polyamine analogs for measuring polyamine transport of the P5B-type ATPases

Marine Houdou[1], Nathalie Jacobs[1], Jonathan Coene[2], Roeland Vanhoutte[2], Mujahid Azfar[1], Veronique Daniëls[3] , Steven Verhelst[2], Peter Vangheluwe[1]

*[1] Laboratory of Cellular Transport Systems; 2Laboratory of Chemical Biology; 3Laboratory for Neurobiology and Gene Therapy, KU Leuven, Belgium.*

*e-mail address presenting author: marine.houdou@kuleuven.be*

Abstract

Polyamines such as putrescine, spermidine and spermine are ubiquitous and physiologically important organic polycations found in every living cell. Polyamines are implicated in a broad range of cellular processes and as a matter of fact, polyamine levels decline with aging whereas polyamine supplementation increases lifespan of model organisms such as mice and fruit fly (1, 2). At the molecular level, polyamine biosynthesis and catabolism pathways are well understood, but a clear knowledge gap remains regarding the molecular characteristics of the mammalian polyamine transport systems (mPTS). Recently, key players of the mPTS were identified belonging to the P5B-type ATPases (e.g. ATP13A2 and ATP13A3) and the Solute Carrier family (e.g. SLC18B1) (3-6). The biochemical characterization of these mPTS typically involves the use of radiolabeled (3H and/or 14C) or fluorescently labeled polyamines to assess polyamine uptake and distribution within the cells. We previously synthesized the clickable Green BODIPY-conjugated polyamine derivatives (7) for the quantification of ATP13A2/3-mediated polyamine uptake (3, 6). Using different chemical synthesis approaches, we here aimed to synthesize additional fluorescent analogs of putrescine, spermidine and spermine to test the effect of different coupling strategies (clickable alkynes, Green BODIPY and BODIPY-FL-T; succinimidyl esters, BODIPY-FL-A) and fluorescent headgroups (BODIPY versus isothiocyanates, FITC). We then characterized these probes in cell models overexpressing the P5B-type ATPases ATP13A2 or ATP13A3 of the mPTS (3, 6). Our results demonstrate that the cellular uptake of all polyamine probes requires the catalytic activity of the P5B ATPases. Amongst the two cell models, ATP13A3 overexpressing cell lines exhibit greater polyamine uptake capacities than ATP13A2 cell models. This observation was confirmed with the use of both radiolabeled polyamines and two of the new green-polyamine probes (BODIPY-FL-A and -FL-T). In addition, the uptake of all BODIPY-conjugated polyamines was consistent in each cellular model and in line with the radiolabeled probes. A higher putrescine and spermidine uptake was found in ATP13A3 cell models whereas higher spermidine and spermine uptake was observed in ATP13A2 cell models, suggesting differences and overlap in polyamine specificities. Conversely, the uptake of FITC-polyamine analogs pointed to similar spermidine and spermine uptake in ATP13A2 and ATP13A3 cell models. We further obtained a better signal to noise ratio with a higher fold change for the uptake of the FITC and new BODIPY-conjugated polyamines (Green, FL-A and FL-T) as compared to the original Green-BODIPY probes, showing that the coupling strategy affects the uptake properties. Since similar fold changes were obtained for the uptake of radiolabeled polyamines and Green-BODIPY polyamines in ATP13A3 cell models, we conclude that the fluorescent tag did not interfere with the uptake. Moreover, the fluorescent probes directly stimulate the ATPase activity of ATP13A2 in vitro suggesting their direct transport by the P5B ATPases. Altogether, our work provides novel fluorescent polyamine analogs to study the cellular polyamine uptake and transport via the P5B ATPases. References 1. T. Eisenberg et al., Induction of autophagy by spermidine promotes longevity. Nat Cell Biol 11, 1305-1314 (2009). 2. T. Eisenberg et al., Cardioprotection and lifespan extension by the natural polyamine spermidine. Nat Med 22, 1428-1438 (2016). 3. S. van Veen et al., ATP13A2 deficiency disrupts lysosomal polyamine export.

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## SESSION 7

*Polyamines in plants and in biotechnological applications*

[L26]

# Comparative genomics assisted mapping of polyamine (PA) biosynthetic pathway in duckweed (*Spirodela polyrhiza*) genome reveals absence of ODC pathway and that PA synthesis genes are differentially regulated during growth, MeJA exposure and salt stress

Rakesh K. Upadhyay[1], Jonathan Shao[2], Autar K. Mattoo[1*]

*[1] Sustainable Agricultural Systems Laboratory and [2]Bioinformatics – North East Area Office, United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, MD 20705-2350, USA.*

*E-mail: autar.mattoo@usda.gov*

**Abstract:** Polyamines (PAs), including putrescine (Put), spermidine (Spd) and spermine (Spm), play key roles in plant growth and development, fruit development and ripening, morphogenesis, and abiotic/biotic stress. Recent advances in genome sequencing led to identification of genes encoding for PA metabolic pathway in some plants. Unfortunately, this is not true for aquatic plants. In this study we performed genome wide mapping of PA metabolic pathway genetic components in duckweed genome (*Spirodela polyrhiza*). We identified PA metabolic encoding genes with 10 PA synthesis genes [*arginase* (*SpARG*), *arginine decarboxylase* (*SpADC1* and *SpADC2*, *agmatine* (SpAIH) *iminohydrolase/deiminase 1*, *N-carbamoyl putrescine amidase (SpCPA)*, three *S-adenosylmethionine decarboxylases (SpSAMDc1, 2, 3)*, two *spermidine synthases/ spermine synthase (SpSPDS1 and SpSPDS2/SPMS]* in the Spirodela polyrhiza genome. Also, we discovered that only ADC pathway encoding genes are present in duckweed genome. None of the annotated locus mapped for ODC genes in released genome assembly and the newest unannotated genome assembly, indicating a possibility of the absence of ODC route of PA synthesis in duckweeds. Hidden Markov Model (HMM) domain analysis established that *SpADC1* is a prokaryotic, arginine type of decarboxylase and may utilize multiple substrates such as arginine/ornithine/lysine. Transcript abundance studies from two clones of duckweeds 7498 and 7003 indicated that all 10 genes are expressed. Salt induced and methyl jasmonate responses of these genes revealed that PA synthesis genes are differentially regulated. This is the first report about the presence of PA metabolic pathway encoding genetic components in duckweeds.

[L27]

## Unraveling the genetics of polyamine metabolism in barley for senescence-related crop improvement

Umesh Kumar Tanwar[1], Ewelina Stolarska[1], Ewelina Paluch-Lubawa[1], Autar K. Mattoo[2], Magdalena Arasimowicz-Jelonek[3], Ewa Sobieszczuk-Nowicka[1]

[1] *Department of Plant Physiology, Faculty of Biology, Adam Mickiewicz University, ul. Uniwersytet Poznańskiego 6, 61-614 Poznań, Poland*

[2] *Sustainable Agricultural Systems Laboratory, United States Department of Agriculture, Agricultural Research Service, Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, MD 20705-2350, USA*

[3] *Department of Plant Ecophysiology, Faculty of Biology, Adam Mickiewicz University, ul. Uniwersytet Poznańskiego 6, 61-614 Poznań, Poland*

*E-mail: ewa.sobieszczuk-nowicka@amu.edu.pl*

Leaf senescence is a hiphly-controlled sequence of events comprising the final stage of development. Understanding its molecular mechanism is important for the improvement of crop yield and postharvest storage. Polyamines (PAs) are low molecular weight organic cations comprising biogenic amines that play multiple roles in plant growth and senescence. Robust PA catabolism can impact the rate of senescence progression in plants. The senescence-dependent PA-mediated multidirectional metabolic crosstalks are important to understand regulation and involvement of PAs in plant death and re-mobilization of nutrients during senescence. Dark-induced leaf senescence (DILS), in the form of severe shading or darkening of leaves, induces leaf senescence similar to that observed during normal plant development. DILS has been utilized as a model to study early and late events in barley leaf senescence. We identified and analyzed the expression of polyamine metabolic pathway genes (HvPMG) in barley, one of the major and important cereals crops, to better understand their role(s) in metabolic and genetic reprogramming during senescence in Gramineae crops. Three S-adenosylmethionine decarboxylases (*HvSAMDCs*), two ornithine decarboxylase, one arginine decarboxylase, one spermidine synthase (*HvSPDS*), two spermine synthases (*HvSPMSs*), five copper amine oxidases (*HvCuAO*) and seven polyamine oxidases (*HvPAOs*), members of the polyamine metabolic pathway gene families (PMGs) were identified and characterized in barley. The DILS model was applied by keeping the barley plants in dark (for 0, 3, 7, 10 days) and gene expression pattern was analyzed for the identified *HvPMG* genes. An early transient response was observed in the expression of *HvSPDS1*, *HvSPMS1* and *HvSPMS2* genes at day-3 in dark, which further decreased as the senescence progressed. The expression of *HvCuAO3* and *HvCuAO7* was upregulated during senescence till day-7, with a decline thereafter. On the other hand, the *HvPAO7* and *HvPAO8* level was upregulated during senescence progression, being highest at day 10. We did not detect *HvPAO2* expression during dark. *HvSPDS1* and *HvSPMS1* were significantly upregulated at day-3 and day-7, comparing control versus dark at each timepoint. Our results not only extend novel findings but also provide valuable information about Gramineae crop development, their stress physiology, and future prospects for genetic improvement programs associated with PAs. This work was supported by the National Science Centre, Poland (project numbers 2018/29/B/NZ9/00734)

[L28]

## Genome-wide exploration of the genetics of biogenic polyamines in barley

Umesh Kumar Tanwar[1], Ewelina Stolarska[1], Ewelina Paluch-Lubawa[1], Autar K. Mattoo[2], Ewa Sobieszczuk-Nowicka[1], Magdalena Arasimowicz-Jelonek[3]

[1] *Department of Plant Physiology, Faculty of Biology, Adam Mickiewicz University, ul. Uniwersytet Poznańskiego 6, 61-614 Poznań, Poland*

[2] *Sustainable Agricultural Systems Laboratory, United States Department of Agriculture, Agricultural Research Service, Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, MD 20705-2350, USA*

[3] *Department of Plant Ecophysiology, Faculty of Biology, Adam Mickiewicz University, ul. Uniwersytet Poznańskiego 6, 61-614 Poznań, Poland*

*E-mail: magdalena.arasimowicz-jelonek@amu.edu.pl*

Barley (*Hordeum vulgare*), a member of the grass family (Gramineae species), is unique among crop plants with importance to agriculture and science. It is one of the major and important crops ranking fourth among grain cereals after maize, wheat, and rice. Barley is also a model experimental system because of its short life cycle and morphological, physiological, and genetic characteristics. We explored the polyamine (PAs) metabolic pathway genes at genome-level in barley to understand their role(s) in plant development and stress adaptation in Gramineae crops. The bioinformatics and functional genomics tools were utilized for genome-wide identification, comprehensive gene features, comparative assessment, evolution and, developmental and stress-related expression analysis of the PA metabolic pathway gene families. We identified three S-adenosylmethionine decarboxylases (*HvSAMDCs*), two ornithine decarboxylase (*HvODCs*), one arginine decarboxylase (*HvADC*), one spermidine synthase (*HvSPDS*), two spermine synthases (*HvSPMSs*), five copper amine oxidases (*HvCuAO*) and seven polyamine oxidases (*HvPAOs*) members of PA metabolic gene (*HvPMG*) family in barley. The *tSPMS/ACULIS5*-like gene was not found. All the identified genes were distributed on all seven chromosomes of barley except for *HvCuAO3* with ChrUn. Gene structure analysis revealed that four genes *HvODC1-2*, *HvPAO5, HvSPDS1* and *HvSAMDC2* were intron-less. Gene duplication analysis showed two tandemly and six segmentally duplicated genes with estimated average divergence time of 82.490 MYA and 51.48 MYA, respectively. Phylogenetic analysis and comparative assessment with other plant species including Arabidopsis, rice, and maize, revealed that PA metabolic pathway is highly conserved in plants. The analysis for potential regulatory mechanisms controlling the gene expression resulted in the prediction of nine *H. vulgare* miRNAs (hvu-miR) target sites in the coding sequences, and 961 putative *cis*-acting elements (CREs) in the promoter region of *HvPMGs*. Expression analysis showed that *HvPMG* genes significantly respond to various stress conditions including heat, cold, salt, osmotic and drought conditions. This novel study is the first to systematically and comprehensively analyze the PAs metabolic pathway gene families in barley.

This work was supported by the National Science Centre, Poland (project number 2018/29/B/NZ9/00734).

[L29]

# Biotechnological and therapeutic applications of nanostructured hybrids of magnetic nanoparticles conjugated with amine oxidase

Andrea Venerando[1], Yuta Kanamori[2], Alberto Macone[2], Massimiliano Magro[1], Enzo Agostinelli[3] [4], Fabio Vianello[1]

[1] *Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, 35020 Legnaro, PD, Italy;* [2] *Department of Biochemical Sciences "A. Rossi Fanelli", SAPIENZA University of Rome, Piazzale A. Moro 5, 00185 Rome, Italy;*

[3] *Department of Sensory Organs, University of Rome "La Sapienza", Policlinico Umberto I, Viale del Policlinico 155, 00161, Rome, Italy.*

[4] *International Polyamines Foundation 'ETS-ONLUS', Via del Forte Tiburtino 98, 00159 Rome, Italy.*

Amine oxidases are key regulator enzymes of polyamine content in cells, influencing cell growth and differentiation. Interestingly, polyamines can be found in higher content in tumour cells than in normal cells and the fine tuning of their cellular levels, as well as of their oxidation products, lead to either cytotoxic or carcinogenic effects. Therefore, the balance of amine oxidases and antioxidant enzymes appear to be crucial for cancer inhibition or progression. To date, several therapeutics exploiting the opportunities provided by nanomaterials have been successfully introduced for the treatment of cancer and other diseases. The primary advantages of these nanostructures reside in their high surface to volume ratio allowing their functionalization with large amounts of both targeting ligands and active compounds, preventing their degradation. In addition, the enhanced permeation and retention (EPR) phenomenon that distinguish solid tumours allows nanoparticles predominantly accumulate into neoplastic tissues. Iron oxide nanoparticles, called surface active maghemite (SAMN) nanoparticles, characterized by peculiar and unusual features have been exploited as smart nano-platforms for the development of bovine serum amine oxidase (BSAO) nano-carrier. In particular, a tannic acid (TA) derivative was prepared (SAMN@TA@BSAO). The biological activity of the as-obtained ternary nanohybrid was studied in cancer cells. Experiments demonstrated that the immobilized enzyme showed increased activity with respect to free enzyme in promoting apoptosis in two neuroblastoma cell lines, namely IMR5 and SJNKP. To note, smart theranostic nanodevice exploiting both EPR and specific interactions between receptors on cancer cell surface and targeting moieties could lead to an attractive outcome of BSAO-based treatment of tumor.

## SESSION 8

*Polyamines metabolism, transport and signal transduction*

[L30]

## Targeting polyamine metabolism to suppress SARS-CoV-2-related disease

Natalia A. Ignatenko[1], Hien Trinh[2], April Wagner[3], David G. Besselsen[3]

[1] *Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ*

[2] *Valley Fiver Center for Excellence, University of Arizona, Tucson, AZ*

[3] *University Animal Care, University of Arizona, Tucson, AZ*

The COronaVIrus disease 2019 (COVID-19) infectious disease is caused by a novel and highly pathogenic virus strain SARS-CoV-2 (Severe acute respiratory virus syndrome coronavirus 2). The SARS-CoV-2 infection causes the lower respiratory tract infection with acute respiratory distress and extrapulmonary organ disfunctions in infected individuals. Treatment strategies that can limit SARS-CoV-2 replication and reduce inflammation associated with the infection should alleviate the disease severity and provide the greatest therapeutic benefit. Polyamines (PA) are naturally occurring organic cations that are essential for growth and development in both prokaryotic and eukaryotic organisms. Many viruses require host polyamines for replication in the infected cells. Previous studies on suppression of polyamine synthesis during infection by other coronaviruses showed promising results in *in vitro* and *in vivo* studies (1). Reducing the intracellular polyamine levels by stimulating their cellular excretion also has limited virus replication (2). The goal of the current study was to test a strategy of lowering polyamine levels by a combination of α-difluoromethylornithine (DFMO) and sulindac, for prevention or treatment of COVID-19 disease. DFMO is an irreversible inhibitor of a key polyamine biosynthetic enzyme ornithine decarboxylase (ODC). Sulindac is a common non-steroidal anti-inflammatory drug (NSAID), which also induces polyamine catabolism. We analyzed the antiviral activity of DFMO, sulindac alone and their combination in SARS-CoV-2 -infected cell culture models using plaque assay and quantified SARS-CoV-2 viral load by qPCR. We found no suppression of plaque development or inhibition of viral propagation in cell lines pretreated or treated with DFMO alone (colon cancer cell line Caco-2 and lung cancer cell line Calu-3 post infection) On a contrary, sulindac alone or DFMO/Sulindac combination were effective in suppressing of the plaque size in a concentration-dependent manner in both prevention and treatments regimes. We also tested the impact of polyamine depletion in a transgenic (*K18-hACE2*) mouse model of COVID-19 disease. The drugs' effects were assessed before infecting (prevention) and after infecting (treatment) by measuring the viral loads, inflammation and clinical phenotype/lethality in infected mice. We found that anti-viral efficacy of DFMO, Sulindac and combination is age- and gender-depended. In young mice (6 weeks old), treatments with the DFMO or Sulindac alone were more effective than DFMO/Sulindac combination. In aged mice (1year old) DFMO/Sulindac combination was more effective in treating the SARS-Cov-2 infection. Female mice in both young and aged experimental groups had 50% increase in the survival rates compared to the treated male mice. Further analysis of this project will determine the effective approach for prevention COVID-19 infection as well as will assess the severity of the viral infection. It is essential to develop new approaches to prevention and treatment of virus outbreaks.

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[L31]

## A novel class of polyamine transporters in health and disease

Sarah van Veen*[1], Norin Nabil Hamouda[1], Mujahid Azfar[1], Stephanie Vrijsen[1], Jianmin Si[2], Chris Van Den Haute[2 3], Veerle Baekelandt[2], Jan Eggermont[1], Shaun Martin[1], Peter Vangheluwe[1]

[1] *Laboratory of Cellular Transport Systems, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium*

[2]*Laboratory for Neurobiology and Gene Therapy, Department of Neurosciences, KU Leuven,* [3]*Leuven Viral Vector Core, KU Leuven*

**sarah.vanveen@kuleuven.be*

For over 40 years, evidence of a complex polyamine transport system (PTS) in mammalian cells has been accumulating, however, the identity of the molecular players in the PTS remained elusive. Many mechanistic models of the mammalian PTS have been proposed, ranging from uptake at the plasma membrane to endocytosis. Our recent publications highlight that members of the P5B ATPase subfamily reside in the endosomal system and are major components of the mammalian PTS. We first demonstrated, in a multi-faceted approach, that the P5B ATPase isoform ATP13A2 is a *bona fide* late endo-/lysosomal polyamine exporter that regulates cellular polyamine uptake[1], representing a landmark in the field. Spermine and spermidine are the major transported substrates of ATP13A2. With fluorescently labeled BODIPY-polyamines, we proved that polyamines first enter the cell via endocytosis, while ATP13A2 exports polyamines from the lysosome to the cytosol. Mutations in ATP13A2 associate with a spectrum of neurodegenerative disorders, including Kufor-Rakeb syndrome, a parkinsonism with dementia, and early-onset Parkinson's disease (PD), and we showed that disease-associated mutations hamper the polyamine transport function of ATP13A2. Dysfunctional ATP13A2 leads to toxic accumulation of polyamines resulting in disturbed lysosomal function, lysosomal rupture and cathepsin B-dependent cell death providing new mechanistic insights in PD pathogenesis. In follow-up studies, we demonstrated that ATP13A2 also mediates mitochondrial polyamine transfer, which provides a potent anti-oxidant response[2], and counteracts α-synuclein aggregation[3], a major hallmark of PD. Together, our studies demonstrated that ATP13A2 is a gatekeeper of endo-/lysosomal functionality and neuronal health, playing a major role in PD. The high conservation of the substrate binding domain in the transmembrane M4 helix implies that also ATP13A3-5 isoforms emerge as components of the mammalian PTS, albeit with a different or overlapping subcellular localization and tissue distribution. Interestingly, ATP13A2-4 are genetically implicated in distinct human diseases: while ATP13A2 plays a role in PD; ATP13A3 is linked to pulmonary arterial hypertension, a rare and fatal cardiovascular disease; and ATP13A4 is genetically linked to neurodevelopmental diseases like specific language impairment and autism. However, direct functional evidence is essential to further validate these genetic findings. Currently, we are investigating the transport properties of ATP13A3/4 to establish whether P5B ATPases present unique or overlapping substrate specificities via complementary biochemical and cellular assays. Our preliminary data indicate that several P5B isoforms stimulate cellular uptake of BODIPY-labeled polyamines. In particular, we demonstrated that mutations in ATP13A3 are responsible for the polyamine transport deficient phenotype of CHO-MG cells. Reintroducing ATP13A3 restored polyamine uptake in CHO-MG cells, whereas ATP13A3 knockdown in WT cells induced polyamine uptake deficiency[4]. In conclusion, we provide strong evidence that P5B ATPase isoforms operate in the mammalian PTS, and with our tools, assays and model systems we are establishing that specific P5B transporters are implicated in distinct diseases.

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[L32]

## Elucidating the role of the lysosomal polyamine exporter ATP13A2 in mitochondrial-lysosomal interplay

Stephanie Vrijsen[1], Chris Van den Haute[1], Chase Chen[2], Yu Chen[2], Ellen Sidransky[2], Veerle Baekelandt[1], Peter Vangheluwe[1]

*[1] KU Leuven, Belgium; [2] NIH NHGRI, U.S.*

*Stephanie.Vrijsen@kuleuven.be*

Loss-of-function mutations in the lysosomal polyamine exporter ATP13A2 (PARK9) lead to a variety of neurodegenerative disorders, including Parkinsonisms (1). At the cellular level, ATP13A2 dysfunction results in lysosomal polyamine accumulation and rupture (2), but also a hampered protection against mitochondrial oxidative stress is observed (3). Interestingly, polyamines transported by ATP13A2 may scavenge reactive oxygen species locally at the level of the mitochondria, as they are redistributed to mitochondria in an ATP13A2-dependent manner (3). To determine the impact of ATP13A2-mediated polyamine transport on the mitochondrial-lysosomal interplay, we here describe a novel method to detect and isolate mitochondria and lysosomes of the same cell population for subsequent omics analysis. We generated SH-SY5Y human neuroblastoma cells with stable co-expression of Tmem192-mEGFP-3xHA (Lyso-IP tag) and TOMM20-mAPPLEx2Strep (Mito-IP tag) for sequential immunoprecipitation of lysosomes and mitochondria. Lyso/Mito-IP fractions collected from the same cells showed strong enrichment of lysosomal and mitochondrial markers, respectively, whereas other organellar markers were absent. Accumulation of lysosomal luminal markers are indicative for intact lysosomes, whereas MitoTracker was able to stain the isolated mitochondria in a mitochondrial membrane potential-specific manner highlighting preserved mitochondrial integrity. Via proteomics analysis, we map differences in the lysosomal/mitochondrial proteome of control *versus* ATP13A2 knockout cells, which provide us insights in the local ATP13A2-mediated signal transduction pathways and mitochondrial/lysosomal interplay. Via metabolomics analysis on Lyso/Mito-IP fractions, we were able to detect organellar polyamine species. Our findings highlight higher lysosomal and lower mitochondrial polyamine levels in ATP13A2 deficient cells, thereby validating our model of polyamine transfer from lysosome to mitochondria. Taken together, sequential pull-down of organelles will provide a useful method to measure organelle specific polyamine content

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## SESSION 9

*Polyamines metabolism in parasites and other microorganisms*

[L33]

## Identification of unique arginine decarboxylase involved in low pH dependent agmatine production in solid-state cultivated *Aspergillus oryzae*

Yui Murakami[1], Wakao Fukuda[1 2], Naoki Akasaka[3], Shinsuke Fujiwara[1 2]

[1] *Department of Bioscience, Graduate School of Science and Technology, Kwansei-Gakuin University, 1 Gakuen Uegahara, Sanda, Hyogo 669-1330, Japan.*

[2] *Department of Biosciences, School of Biological and Environmental Sciences, Kwansei-Gakuin University, 1 Gakuen-Uegahara, Sanda, Hyogo, 669-1330 Japan*

[3] *Laboratory for Circular Bioeconomy Development,Office of Society Adademia Collaboration for Innovation, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan*

*e-mail address of presenting author: fujiwara-s@kwansei.ac.jp*

Agmatine, a natural polyamine produced from arginine by arginine decarboxylase, was firstly discovered in 1910, but its physiological significance was unclear for a century. The recent rediscovery of agmatine as an endogenous ligand for $a_2$-adrenergic and imidazoline receptors in the mammalian brain suggests that this amine may be a promising therapeutic agent for treating a broad spectrum of central nervous system-associated diseases. In the past two decades, numerous preclinical and several clinical studies have demonstrated its pleiotropic modulatory functions on various molecular targets related to neurotransmission, nitric oxide synthesis, glucose metabolism, polyamine metabolism, and carnitine biosynthesis, indicating potential for therapeutic applications and use as a nutraceutical to improve quality of life (1,2). An enzymatic activity of arginine decarboxylase which produces agmatine from arginine was low in mammals, suggesting that a large portion of the agmatine is supplemented from diets and gut microbiota. It was noteworthy some fermented foods by *Aspergillus oryzae* contain relatively large amounts of agmatine even though *A. oryzae* lacks L-arginine decarboxylase (ADC) orthologs in the genome. Homogenate from a solid-state culture exhibited a maximum ADC activity at pH 3.0 at 30°C; that from a submerged culture exhibited an extremely low activity under all conditions tested (3). These observations indicated that efficient agmatine production is achieved by an unidentified low pH-dependent ADC induced during solid-state cultivation of *A. oryzae* (4). Recently, we purified natural ADC from *A. oryzae* hypha obtained in solid-state cultivation and ADC was identified by LC-MS and sequence analysis. The newly identified ADC possessed the typical feature of pyruvyl dependent decarboxylase in amino acid sequence. Recombinant form of the ADC was expressed in *Escherichia coli* cells and purified to homogeneity, showing a low pH-dependent ADC activity as natural ADC did.

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[L34]

## Optimization of Potent and Specific Trypanothione Reductase Inhibitors: A Structure-Based Drug Discovery Approach

Gianni Colotti[a*] , Theo Battista[a b ‡], Stefano Federico[c ‡], Simone Brogi[d], Luca Pozzetti[c], Stefania Butini[c], Anna Ramunno[e], Eleonora Fiorentino[f], Stefania Orsini[f], Trentina Di Muccio[f], Annarita Fiorillo[a g], Cécile Exertier[a], Sandra Gemma[c], Giuseppe Campiani[b †], Andrea Ilari[a † *]

[a] *Institute of Molecular Biology and Pathology (IBPM) of the National Research Council of Italy (CNR), c/o Department of Biochemical Sciences, Sapienza University of Rome, Piazzale A. Moro 5, 00185, Roma, Italy*

[b] *Department of Chemical and Pharmaceutical Sciences, University of Trieste Via L. Giorgieri, 1 34127 Trieste (Italy)*

[c] *Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via Aldo Moro 2, 53100, Siena, Italy*

[d] *Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126 Pisa, Italy*

[e] *Department of Pharmacy/DIFARMA, University of Salerno, via Giovanni Paolo II 132, 84084, Fisciano, Salerno, Italy*

[f] *Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy*

[g] *Department of Biochemical Sciences, Sapienza University of Rome, Piazzale A. Moro 5, 00185, Roma, Italy*

**Keywords:** Trypanothione, Trypanothione Reductase, *Leishmania*, 2-nitrothiophene heterocyclic system

**Abstract.** Leishmaniasis, caused by protozoa from over 20 *Leishmania* species, affects up to 1 million people every year. The current therapeutic arsenal against *Leishmania* is largely inadequate and there is an urgent need for better drugs. Trypanothione reductase (TR) represents a druggable target since it is essential for the parasite and not shared with the human host. Starting from LeishBox, a set of 192 best antileishmanial compounds identified by GlaxoSmithKline, we identified as best hit targeting TR, A1/7 (compound 1) endowed with a sub-micromolar inhibitory potency against TR of *L. infantum* (LiTR: IC50 = 0.52 ± 0.14 μM) and a good selectivity index toward glutathione reductase (hGR), i.e. the closest human enzyme involved in the detoxification of ROS. We solved the high resolution crystal structure of TR in complex with compound 1, demonstrating that this inhibitor binds into a sub-pocket placed at the dimeric interface lined by the residues of the 397-403 β-turn, by the catalytic residues H461, E466, and E467 and K61', placed on the two-fold symmetry-related subunit. Based on these results, we designed and synthesized a series of compounds that were tested for their inhibitory activity on LiTR and in parallel for their activity against hGR enzyme. SAR analysis showed that the 2-nitrothiophene heterocyclic system, which forms a network of H-bonds with K61' and the NH of L399, is fundamental for the inhibitory activity of this class of compounds, whereas the replacement of the methoxy-groups of compound 1 led to the synthesis of more potent derivatives. Our studies could pave the way for novel therapeutic approaches not only against leishmaniasis but also against other Trypanosomatidae, due to the structural similarity of TR enzymes.

# POSTERS

**[P01]** T. Tahara, L. Di Magno, S. Coni, D. Ivy, L. Rutigliano, R. Pellegrino, A. Montella, G. Canettieri, E. Agostinelli
**Anti-cancer effect of spermidine inducing autophagy-dependent cell death**

**[P02]** Sarah van Veen, Chris Van Den Haute, Veerle Baekelandt, Jan Eggermont, Matthew Holt, Peter Vangheluwe
**Quest for the transported substrate of ATP13A4, a putative polyamine transporter linked to neurodevelopmental disorders**

**[P03]** Holly Moots, Houssine Ikhlef, and Otto Phanstiel
**Development of LAT-1 efflux agonists to control pancreatic cancer cell growth**

**[P04]** Weiman Gao, Lin Xiao, Ruby Pandher, Mawar Karsa**,** Emma Ronca, Angelika Bongers**,** Murray D. Norris Michelle Haber, and Klaartje Somers
**Polyamine transporter ATP13A3 is a novel therapeutic target in neuroblastoma**

**[P05]** V.L. Filonov, M.A. Khomutov, A.V. Sergeev, E.S. Gromova, A.R. Khomutov
**Phosphorus analogues of AdoMet and AdoHCy: Synthesis and interaction with AdoMet decarboxylase and DNA methyltransferase Dnmt3a**

**[P06]** Rilievo G., Cecconello A., Magro M., Molinari S., Venerando A. Agostinelli E., Vianello F.
**Functional nanocarrier for bovine serum amine oxidase**

**[P07]** Maria Luisa Di Paolo, Andrea Milelli, Lisa Dalla Via, Giulia Nordio, Giorgio Cozza and Anna Minarini
**From an "old" polyamine analog toward novel derivatives to target monoamine oxidases**

**[P08]** Alessandro Montella[1], Silvia Grancara[2], Lisa Dalla Via[3], Raffaele Pellegrino[1], Tomoaki Tahara[1 4] Lavinia Rutigliano[1 4], Gianluca Canettieri[4 5 6], Antonio Toninello[2] and Enzo Agostinelli[1 5]
**Spermine enzymatic oxidation products induce mitochondrial alterations in human cancer cells lower in presence of glucose, detected by mass spectroscopy analysis**

**[P09]** Cristian Fiorucci, Giulia Baroli, Paolo Mariottini,Manuela Cervelli
**Molecular mechanisms of polyamines in comorbidity of mental disorders**

**[P10]** Marianna Nicoletta Rossi, Roberta Ceci, Guglielmo Duranti, Stefano Giuliani, Ivan Dimauro, Stefania Sabatini, Paolo Mariottini, Manuela Cervelli
**Spermidine affects gene expression profiles and redox imbalance in C2C12 myoblasts treated with hydrogen peroxide**

**[P11]** Rosa Bordone, Devon Michael Ivy, Fiorella Di Pastena, Laura Di Magno, Sonia Coni, Gianluca Canettieri
**A gene editing approach reveals that myc ires sequence does not mediate cap-independent translation and resistance to stress conditions**

**[P12]** EA VanSickle[1 2], J Michael[1 2], M Vipond[1 2], S Rajasekaran[1 2 3], JW Prokop[3], AS Bachmann[2 3], CP Bupp[1 2 3]
**Bachmann-bupp syndrome – emerging cases of an ultra-rare polyamine disorder**

**[P13]** Sophia Zaletok[1], Oleg Klenov[1], Veronika Bentrad[1], Natalia A. Ignatenko[2], Yuriy Vitruk[3], Eduard Stakhovsky[3]
**Polyamines – Tissue Markers For The Differential Diagnosis And Assessment Of The Aggressiveness Of Prostate Cancer**

[P01]

## Anti-cancer effect of spermidine inducing autophagy-dependent cell death

T. Tahara[1 2], L. Di Magno[1], S. Coni[1], D. Ivy[1], L. Rutigliano[1 2], R. Pellegrino[2], A. Montella[2], G. Canettieri[1 3 4], E. Agostinelli[2 3].

[1] *Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena 291, 00161 Rome, Italy*

[2] *Department of Sensory Organs, Sapienza University of Rome, Policlinico Umberto I, Viale del Policlinico 155, 00161 Rome, Italy*

[3] *International Polyamines Foundation 'ETS-ONLUS', Via del Forte Tiburtino 98, I-00159 Rome, Italy*

[4] *Istituto Pasteur, Fondazione Cenci-Bolognetti, Sapienza University of Rome, Viale Regina Elena 291, 00161 Rome, Italy*

Polyamine synthesis has been regarded as the candidate target for cancer therapy since many cancers contain plenty of polyamines compared to non-cancer cells. On the other hand, toxic substances such as aldehyde(s) or acrolein and $H_2O_2$ generated by SSAT and amino oxidase in the medium have been considered the main component of polyamine cytotoxicity *in vitro*. Recent research reported the protective role of spermidine in carcinogenesis *in vivo*[1] . However, there is still room for investigation on the mechanism of cytotoxicity of polyamines represented by spermidine.

Here, I report the possibility of a new cytotoxic mechanism by exogenous spermidine. In the polyamine depleted state induced with DFMO, SPD has a dose-dependent bidirectional effect of contributing to and inhibiting cell proliferation. When polyamine synthesis was not inhibited, cell proliferation was inhibited at SPD concentrations that contributed to proliferation during polyamine depletion. This SPD cell proliferation inhibition was effective against a wide range of cancer cell lines. Interestingly, this polyamine-induced cell proliferation did not show significant activation of Caspase 3 and PARP, suggesting that Caspase and PARP-independent cell death other than apoptosis were involved. Furthermore, the addition of chloroquine, an autophagy inhibitor, inhibited the suppression of cell proliferation of polyamine, suggesting that this cell death is at least partially autophagy-dependent cell death while LC3B-II expression was independent of SPD treatment.

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[P02]

## Quest for the transported substrate of ATP13A4, a putative polyamine transporter linked to neurodevelopmental disorders

Sarah van Veen*[1], Chris Van Den Haute[2 3], Veerle Baekelandt[2], Jan Eggermont[1], Matthew Holt[4], Peter Vangheluwe[1]

[1]*Laboratory of Cellular Transport Systems, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium*

[2]*Laboratory for Neurobiology and Gene Therapy, Department of Neurosciences, KU Leuven, Leuven, Belgium*

[3]*Leuven Viral Vector Core, KU Leuven, Leuven, Belgium*

[4]*Laboratory of Synapse Biology, i3S, University of Porto, Porto, Portugal*

**sarah.vanveen@kuleuven.be*

Neurodevelopmental disorders constitute a serious public health challenge in modern society due to their high prevalence, their complex and heterogeneous pathophysiology, as well as their high health care costs and impact on quality of life. Neurodevelopmental disorders affect more than 3% of children worldwide and have been linked to mutations at over 1000 loci. ATP13A4 is a promising candidate gene that has been associated with specific language impairment, autism spectrum disorder (ASD) and psychiatric diseases. A chromosomal inversion disrupted the ATP13A4 gene in two specific language impairment patients, without further cognitive deficiencies. Moreover, whole-exome sequencing identified a heterozygous ATP13A4 E646D variant in three study participants with childhood apraxia of speech, a rare and severe motor speech disorder. Furthermore, an autism susceptibility locus was mapped on chromosome 3q25-27, near to the location of ATP13A4, in a genome-wide screen for ASD. Subsequent DNA sequencing of thirty patients revealed an ATP13A4-E646D sequence variant in six study participants and an A356V substitution in another patient. In addition, ATP13A4 gene deletions were reported in schizophrenia patients. Altogether, ATP13A4 may be implicated in several neurodevelopmental disorders, but its biological and (patho)physiological role remains unknown. ATP13A4 is an endosomal orphan P-type ATPase transporter that together with ATP13A2 belongs to the P5B-type ATPase subfamily. We recently demonstrated that ATP13A2 is a late endo-/lysosomal polyamine transporter involved in neurodegeneration[1]. Polyamines, such as putrescine, spermidine and spermine, are low-molecular-weight polycations that are highly abundant in the brain and crucial for normal brain function. Polyamines influence several processes that are implicated in the pathophysiology of ASD, epilepsy and schizophrenia. Indeed, polyamines are potent anti-oxidants, exert anti-inflammatory actions, support protein synthesis and folding, membrane stability, signal transduction, neurotransmission, autophagy, cell growth and proliferation. ATP13A2 and ATP13A4 share a high sequence similarity in the substrate binding site suggesting that ATP13A4 may transport the same or closely related polyamines. Our preliminary data indicate that overexpression of ATP13A4 WT in H4 human neuroglioma cells promotes the uptake of BODIPY-labelled polyamines and confers sensitivity to polyamine toxicity, as demonstrated by cell viability and cell death assays. This phenotype is not observed in cells overexpressing the catalytically inactive D486N mutant, which shows that polyamine transport activity plays a role in these phenotypes. Following inhibition of polyamine synthesis, we do not see a difference in cell viability between the different H4 cell lines, whereas ATP13A4 transport activity sensitizes cells to SAT1 inhibition. In addition, we found a synergistic effect of spermidine and SAT1 inhibition on cytotoxicity. We conclude from these data that cells expressing ATP13A4 rely more on polyamine catabolism, most likely as a consequence of increased intracellular polyamine levels. In conclusion, our preliminary findings strongly indicate that, like ATP13A2, ATP13A4 transports polyamines into the cytosol of the cell. Future in-depth characterization of both the cytosolic and organellar impact of ATP13A4's transport function will be instrumental in understanding its role in the brain and may validate ATP13A4 as a candidate therapeutic target for neurodevelopmental disorders.

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[P03]

## Development of LAT-1 efflux agonists to control pancreatic cancer cell growth

Holly Moots, Houssine Ikhlef, Otto Phanstiel

*12722 Research Parkway, Department of Medical Education, University of Central Florida College of Medicine, Orlando, Florida, USA 32826.*

*Otto.Phanstiel@ucf.edu*

**Abstract.** The large amino acid transporter 1 (LAT1) is the primary transporter of methionine, leucine, and phenylalanine into human cells. We have discovered a novel agent, compound ***255***, which facilitates the efflux of these key amino acids via LAT1. Compound ***255*** provides the interesting ability to limit methionine inside human cells in a dose dependent fashion. Methionine is an essential amino acid, which can be converted to decarboxylated *S*-adenosylmethionine (dc-SAM) to enable spermidine and spermine biosynthesis via spermidine synthase and spermine synthase, respectively. Here we show in L3.6pl human pancreatic cancer cells that loss of intracellular methionine via LAT1 efflux generates decreased intracellular levels of spermidine and spermine, but not putrescine. This approach provides a novel way to limit levels of the higher polyamines without affecting putrescine levels.

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[P04]

## Polyamine transporter ATP13A3 is a novel therapeutic target in neuroblastoma

Weiman Gao[1 2], Lin Xiao[1 2], Ruby Pandher[1 2], Mawar Karsa[1 2], Emma Ronca[1], Angelika Bongers[1], Murray D. Norris[1 2], Michelle Haber[1 2], Klaartje Somers[1 2]

[1.] *Children's Cancer Institute, Lowy Cancer Research Centre, UNSW Sydney, Sydney, NSW, Australia.*

[2.] *School of Women's and Children's Health, UNSW Sydney, Sydney, NSW, Australia.*

*Email address of presenting author: lgao@ccia.org.au*

**Introduction**: Neuroblastoma is a cancer derived from neural crest progenitor cells of the peripheral nervous system, most commonly arising in the adrenal gland. It is the most common extracranial solid tumour in children and the most frequently diagnosed neoplasm during infancy. High-risk neuroblastoma has dismal survival rates of less than 50%. Neuroblastoma tumours are characterised by elevated levels of polyamines as well as by upregulation of polyamine synthesis and polyamine uptake from the extracellular environment. We recently demonstrated that neuroblastoma cells, when treated with DFMO, a drug that inhibits polyamine synthesis enzyme ODC1, can rescue themselves from DFMO treatment by upregulating polyamine uptake from the extracellular environment, a process efficiently inhibited by the novel drug AMXT 1501. While, based on our findings, the combination of DFMO and AMXT 1501 is currently being progressed into clinical trial for neuroblastoma patients, very little is known about the polyamine uptake system and polyamine transporters in neuroblastoma.

**Aims:** This study aims to discover novel targets for polyamine blocking therapy by characterising the polyamine transport system.

**Methods and results:** We found that increased expression of the putative polyamine transporter ATP13A3 is associated with worse survival in a neuroblastoma patient cohort (n=498), suggesting a role for this transporter in disease progression. Silencing of ATP13A3 significantly inhibits polyamine uptake in neuroblastoma cells and prevents DFMO-induced compensatory polyamine uptake similarly to AMXT 1501. Moreover, silencing of ATP13A3 expression sensitises neuroblastoma cells to DFMO, suggesting a role for ATP13A3 as an additional therapeutic target within the polyamine pathway.

**Conclusion:** We identified ATP13A3 as an important polyamine transporter in neuroblastoma and future investigations will further elucidate its potential as a therapeutic target in neuroblastoma animal models.

[P05]

# Phosphorus analogues of AdoMet and AdoHCy: Synthesis and interaction with AdoMet decarboxylase and DNA methyltransferase Dnmt3a

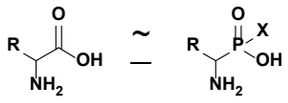
V.L. Filonov[1], M.A. Khomutov[1], A.V. Sergeev[2], E.S. Gromova[2], A.R. Khomutov[1]

[1]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia*

[2]*Chemistry Department, Lomonosov Moscow State University, Moscow, Russia*

*e-mail: alexkhom@list.ru*

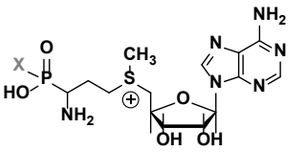
The replacement of the carboxyl group of amino acids with an acidic phosphorus-containing group gives rise to a big family of the compounds of the general formula:




X= H, HO, R', OR'', NHR'''

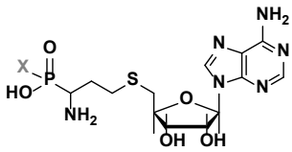
This type of substances is sterically different and has ionization constants different from amino acids, including the pKa of the $H_2N$-group. However, aminophosphonates are capable to interfere with amino acid metabolism both at the level of the isolated enzymes and at the organism's level, and their action can be manifested at various stages of metabolism.

Most studied are aminophosphonic acids (X= OH) and their derivatives (X= R', OR", NHR"'). The phosphorus-containing group is considered as a mimetic of the intermediate tetrahedral state of the carboxyl group and many aminophosphonates are effective inhibitors of the corresponding enzymes. Aminophosphinic acids (X=H) are different from aminophosphonic acids, since their single-charged phosphorus containing group is a flatted tetrahedron, which in some cases models well a planar single-charged carboxyl group. Therefore, having penetrated in the cell, some phosphinic analogues of amino acids can act not only as such, but also to undergo substrate-like transformations leading to the formation of new biologically active metabolites.




X=H: AdoMet-$P_H$

X=OH: AdoMet-*P*




X=H: AdoHCy-$P_H$

X=OH: AdoHCy-*P*

Phosphonic (X=OH) and phosphinic (X=H) analogues of *S*-adenosylmethionine and *S*- adenosylhomocysteine were synthesized. The interaction of *S*-adenosylmethionine decarboxylase with AdoMet-$P_H$ and AdoMet-*P* and the interaction of phosphonic and phosphinic analogues of *S*-adenosylmethionine and *S*-adenosylhomocysteine with DNA methyltransferase Dnmt3a are discussed.

This work was supported by the Russian Science Foundation grant no. 22-14-00291.

[P06]

## Functional nanocarrier for bovine serum amine oxidase

Rilievo G.[a], Cecconello A.[a], Magro M.[a], Molinari S.[b], Venerando A.[a], Agostinelli E.[c d], Vianello F.

[a]*Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, 35020, Legnaro (PD), Italy;*

[b]*Department of Geosciences, University of Padua, Via Gradenigo 6, 35131 Padova, Italy;*

[c]*Department of Sensory Organs, University of Rome "La Sapienza", Policlinico Umberto I, Viale del Policlinico 155, 00161, Rome, Italy;*

[d]*International Polyamines Foundation 'ETS-ONLUS', Via del Forte Tiburtino 98, 00159 Rome, Italy.*

Protein-nanoparticle hybrids represent novel entities with different biological properties with respect to parent bio-macromolecules. Herein, serum amine oxidase (BSAO) was immobilized onto a core-shell magnetic nanomaterial constituted of surface active maghemite nanoparticles (SAMNs, the core) modified with tannic acid (TA, the shell), leading to a novel functional ternary hybrid (SAMN@TA@BSAO), which was characterized by dynamic light scattering and transmission electron microscopy. The kinetic characterization showed that enzyme immobilization shifted the optimum pH from 7.0 to 5.0. Infrared spectroscopy and circular dichroism spectroscopies indicated that, differently from native enzyme, the secondary structure of immobilized BSAO was sensitive to pH. Due to its catalytic activity on polyamine oxidation, the SAMN@TA@BSAO ternary hybrid can be proposed for generating cytotoxic products, such as aldehydes and $H_2O_2$, following its introduction in tumour cells. The present example supports the nascent knowledge that protein-nanoparticle conjugation can represent a key for the modulation of biological functions.

[P07]

## From an "old" polyamine analog toward novel derivatives to target monoamine oxidases

Maria Luisa Di Paolo[1], Andrea Milelli[2], Lisa Dalla Via[3 4], Giulia Nordio[3 4], Giorgio Cozza[1] and Anna Minarini[5]

[1] *Department of Molecular Medicine, University of Padova, Padova, Italy, marialuisa.dipaolo@unipd.it*

[2]*Department for Life Quality Studies, University of Bologna, Rimini Campus, Rimini, Italy*

[3]*Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy*

[4] *Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali (INSTM), via G. Giusti 9, 50121 Firenze (Italy).*

[5] *Department of Pharmaceutical Sciences, Alma Mater Studiorum-University of Bologna, Bologna, Italy*

Polyamine analogues (PA) are extensively investigated for their potential pharmacological applications as anticancer, anti-parasitic, anti-bacterial, and anti-neurodegenerative agents. Indeed, polyamine structures can bind to various targets and their affinity and selectivity may be fine-tuned by inserting appropriate substituents and varying the methylene chain lengths of the polyamine backbone (Minarini et al., 2010). Our recent studies highlighted that some symmetric polymethylene tetraamines can act as inhibitors of monoamine oxidases (MAO) (Bonaiuto et al 2012, Bonaiuto et al 2013; Di Paolo et al 2019), which are "old" and well-known targets of anti-neurodegenerative drugs (mainly the isoform MAO B) and antidepressant therapies (mainly the isoform MAO A) (Bejl T al. 2021). Additionally, recent findings suggest that MAOs could be also potential targets in anticancer therapies. (Alianabi et al. (2021). In our studies, we found that benextramine, a tetraamine disulfide α-adrenergic antagonist, able to hit additional targets involved in neurodegeneration (Melchiorre et al. 2003), acts also as irreversible inactivator of MAOs, mainly MAO A (Di Paolo et al.2019), while ELP 12, a methoctramine derivative with an inner less flexible dipiperidine moiety, endowed with acetylcholinesterase inhibitory activity, might be a good lead to develop reversible MAO B inhibitors (Bonaiuto et al, 2012). In this study we explored the effect of the replacement/substitution of the inner dipiperidine moiety of ELP 12, with "aromatic moieties" and five novel derivatives were designed and synthtized. The inhibitory potency and selectivity of these novel derivatives towards the two MAO isoforms was evaluated. By a kinetic approach, the inhibition constant value and mechanism of inhibition were determined. Additionally, the potential cytotoxicity of the tested compounds was evaluated investigated by an *in vitro* assay on human tumor cell lines. Among the novel PA, ELP 24 results to be the most promising compound, being endowed of an increased inhibitory potency (Ki in the microMolar range) for MAOs in comparison to that of the "lead" ELP12. Optimization studies are now in progress with the aim to modify the structure of the described compounds in order to improve their affinity and selectivity for potential pharmacological application.

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[P08]

## Spermine enzymatic oxidation products induce mitochondrial alterations in human cancer cells, lower in presence of glucose, detected by mass spectroscopy analysis

Alessandro Montella[1], Silvia Grancara[2], Lisa Dalla Via[3], Raffaele Pellegrino[1], Tomoaki Tahara[1 4] Lavinia Rutigliano[1 4], Gianluca Canettieri[4 5 6], Antonio Toninello[2], Enzo Agostinelli[1 5]

[1] *Department of Sensory Organs, Sapienza University of Rome, Policlinico Umberto I, Viale del Policlinico 155, I-00161 Rome, Italy*

[2] *Department of Biomedical Sciences, University of Padua, I-35131 Padua, Italy*

[3] *Department of Pharmaceutical and Pharmacological Sciences, University of Padua, I-35131 Padua*

[4] *Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena 291, 00161 Rome, Italy*

[5] *International Polyamines Foundation 'ETS-ONLUS', Rome, Italy*

[6] *Pasteur Institute, Fondazione Cenci-Bolognetti, Rome, Italy*

In situ formation of cytotoxic metabolites by an enzyme-catalyzed reaction is a recent approach in cancer chemotherapy. By a clonogenic cell survival assay performed in PBS-1% BSA, we demonstrated that multidrug resistant human colon adenocarcinoma cells (LoVo DX) are more sensitive than the corresponding wild type cells (LoVo WT) to hydrogen peroxide and aldehydes, the products of bovine serum amine oxidase (BSAO)-catalyzed oxidation of spermine. The same cytotoxic effect was observed on both neuroblastoma cancer cells SJNKP and IMR5 Myc overexpressed. Hydrogen peroxide resulted to be the metabolite mainly responsible for the loss of cell viability, although spermine-derived aldehyde(s) induced some cytotoxicity (less than 20%). A decreasing of cytotoxicity was observed in all tumor cell lines when the experiments were carried out in presence of glucose 10mM in the incubation mixture (PBS-1%BSA). Glucose mainly protected tumor cells against the cytotoxic effect due to $H_2O_2$ generated by the enzymatic reaction. Interestingly, the treatment of human tumor cells with spermine and bovine serum amine oxidase (BSAO), induces cytotoxicity and strong mitochondria alterations as evidenced by electron microscopy observations. Biochemical analyses on isolated tumor cell mitochondria demonstrate membrane potential collapse, matrix swelling, oxidation of pyridine nucleotides and glutathione, and release of endogenous cations. Again, immunological experiments evidence the release of pro-apoptotic factors. Indeed, proteomic analyses by mass spectrometry, always on tumor cells, show that among the identified 721 proteins, all the detected mitochondrial ones, probably belonging to the transition pore, are downregulated by confirming their involvement in the transition pore opening. So, it is to propose that the cytotoxic mechanism is strongly related to mitochondrial dysfunctions. In conclusion, these findings suggest that toxic products formed by the oxidation of spermine by BSAO could be a power tool in the development of new anticancer therapies.

[P09]

## Molecular mechanisms of polyamines in comorbidity of mental disorders

Cristian Fiorucci[1], Giulia Baroli[1], Paolo Mariottini[1] Manuela Cervelli[1 2]

[1] *Department of Science, University of "Roma Tre", Rome.*

[2] *Neurodevelopment, Neurogenetics and Molecular Neurobiology Unit, IRCCS Fondazione Santa Lucia, Via del Fosso di Fiorano 64, 00143 Rome, Italy*

*Email of presenting author: cristian.fiorucci@uniroma3.it*

Spermine oxidase (SMOX) is an enzyme that catalyses polyamines and contains FAD. Endogenous polyamines are necessary for cell growth, proliferation, regeneration, and differentiation in eukaryotic cells. Three enzymes, spermidine/spermine-N1-acetyltransferase, acetylpolyamine oxidase, and SMOX, work together to catabolize polyamines. Spermine oxidase specifically oxidises spermine to produce $H_2O_2$, spermidine, and 3-aminopropanal, and it is implicated in drug response, apoptosis, and the pathogenesis of many diseases, including cancer. We created a Dach-SMOX transgenic mouse line that overexpresses SMOX in the cerebral cortex. We demonstrated that Dach-SMOX mice were more susceptible to kainate-induced epileptic seizures and excitotoxic brain damage. The results obtained in the behavioural and molecular characterization of Dach-SMOX mice show that they also display an anxious phenotype. In particular we found an altered glutamatergic transmission and expression of some serotonin receptors and transporters. The significant comorbidity between anxiety and epilepsy strongly suggests that they share a common background in the Central Nervous System. Polyamines are protective molecules that defend the brain against the development of epilepsy and mental diseases and are produced by various types of neurons. In the present study we show that Dach-SMOX mouse model could contribute to understand the molecular mechanisms by which polyamines are involved in the aetiology of anxiety and epilepsy. To sum up, polyamine metabolism may be a potential clinical target for prevention and treatment of these brain diseases with huge impact on quality of patient life.

[P10]

## Spermidine affects gene expression profiles and redox imbalance in C2C12 myoblasts treated with hydrogen peroxide

Marianna Nicoletta Rossi[1], Roberta Ceci[2], Guglielmo Duranti[2], Stefano Giuliani[1], Ivan Dimauro[3], Stefania Sabatini[2], Paolo Mariottini[1], Manuela Cervelli[1 4]

[1] *Department of Science, "Department of Excellence 2018–2022", University of Rome "Roma Tre", 00146 Rome, Italy;*

[2] *Laboratory of Biochemistry and Molecular Biology, Department of Movement, Human and Health Sciences, Università degli studi di Roma "Foro Italico", 00135 Rome, Italy*

[3] *Laboratory of Biology and Genetics, Department of Movement, Human and Health Sciences, Università degli studi di Roma "Foro Italico", 00135 Rome, Italy.*

[4] *Neurodevelopment, Neurogenetics and Molecular Neurobiology Unit, IRCCS Fondazione Santa Lucia, Via del Fosso di Fiorano 64, 00143 Rome, Italy*

*Email of presenting author: mariannanicoletta.rossi@uniroma3.it*

Skeletal muscle cells do not have locomotive function only, but they exert endocrine functions and have remarkable regenerative properties. Through their capacity to secrete myokines, skeletal muscle is involved in maintaining the energy balance of the whole organism. A reduction or loss of the muscle regenerative potential characterizes several physiopathological conditions, like atrophy (muscle mass loss) and cachexy and several degenerative muscular diseases like Duchenne muscular dystrophy (DMD) and amyotrophic lateral sclerosis (ALS). Reactive oxygen species (ROS), such hydrogen peroxide ($H_2O_2$), exert a critical regulatory role on skeletal muscle function. Moderate ROS levels are critical for cell signaling, for gene expression and are necessary for muscle growth. On the contrary, high levels of ROS increase myoblasts cell death and worsen muscle repair in aging and in degenerative muscular diseases. There are several evidence linking Spermidine (Spd) to skeletal muscle homeostasis, but the effects of Spd on redox balance in skeletal muscle cells is a field quite unexplored so far. We evaluated the role of Spd in murine C2C12 myoblasts treated with a low dose of $H_2O_2$. In particular, we addressed myoblasts viability; redox status; and polyamine metabolism. $H_2O_2$ exposure induced increased cell death, redox status imbalance and provoked perturbation in polyamine metabolism. Spd treatment was able to increase cell number and to reduce cell death induced by the $H_2O_2$ insult. Moreover, Spd was able to mitigate the redox imbalance. Finally, $H_2O_2$ treatment increases the expression levels of the *SAT1* and *SMOX* genes while, the addition of Spd reverts the effects of $H_2O_2$ treatment on *SMOX* and *SAT1* gene expression. In conclusion, our data suggest that Spd has a protective role in skeletal muscle by restoring redox balance and counteracting oxidative insults.

[P11]

# A gene editing approach reveals that myc ires sequence does not mediate cap-independent translation and resistance to stress conditions

Rosa Bordone[*1], Devon Michael Ivy[*1], Fiorella Di Pastena[2], Laura Di Magno[1], Sonia Coni[1] Gianluca Canettieri[1]

[1] *Sapienza University of Rome, Department of molecular medicine, Rome (RM), Italy*

[2] *McMaster University, Faculty of Health Sciences, Dep. Of Medicine, Hamilton (ON), Canada*

*rosa.bordone@uniroma1.it*

## Introduction

The Internal Ribosome Entry Site (IRES) is a region located in many viral transcripts to allow the translation of viral proteins when cellular cap-dependent protein synthesis is inhibited by the infection (1). While in the viral context the existence of IRESes is widely accepted, the possibility that mammalian mRNA could undergo IRES mediated translation is still debated. In the past years, several authors identified putative IRES sequences in the 5' untranslated regions of mammalian messenger RNA, particularly in genes involved in cell viability, proliferation, and cell cycle regulation. A well characterized mammalian IRES resides in the 5'UTR of the proto-oncogene MYC (2). It was hypothesized that, when the cell is subjected to stress conditions, general protein synthesis is inhibited and, to escape death, cells activate different mechanisms including IRES-mediated translation of key transcripts, to maintain or even increase their translation. In this view, IRES mediated translation of some transcripts, including MYC, could also be exploited by cancer cells to survive to the unfavorable, stressing conditions occurring during cancer development, such as metabolic, replication or ER stresses. In the last decade, however, many authors argued against the experimental approach used to identify mammalian IRESes, especially the widely used bicistronic reporter assay (3). Aim of the present study was to characterize MYC IRES function using a reliable and physiological approach of genome editing, to give a conclusive answer to this relevant question.

## Methods

To address the role of MYC IRES, we generated a cell line using the CRISPR-Cas9 technique to remove the region previously identified as the IRES sequence. The cells obtained (ΔIRES) were subjected to stress conditions to evaluate MYC levels and cell viability.

## Results

We first tested the ability of the IRES region to mediate the cap-independent translation of MYC using in vitro transcription of a reporter mRNA fused with the IRES with or without cap. After transfection of the resulting transcripts, we observed that, in the absence of cap, luciferase translation was completely abrogated even in the presence of the putative IRES. This data was confirmed by lentiviral mediated knockdown of the cap-binding factor eIF4E and by interfering mTOR to activate 4EBP mediated sequestration of eIF4E. In all cases tested, MYC protein synthesis was abolished to the same extent in ΔIRES and control cells, regardless the presence of the IRES region. We then exposed ΔIRES cells to several known inductors of cellular stress to test the ability of our cells to survive and maintain MYC expression with or without the IRES. We started testing Endoplasmic Reticulum stress (ER stress) and Genotoxic stress previously described to activate MYC IRES-dependent translation. Conversely to what expected, ΔIRES cells showed the same sensitivity to the drugs tested and same MYC protein levels. Therefore, we decided to evaluate the response of our cells after induction of oxidative and metabolic stress. Also in these cases ΔIRES cells were as sensitive as control cells to the stress conditions and expressed MYC at equal levels.

## Conclusions

Overall, we used a more physiological approach to demonstrate that the region previously identified as MYC IRES is not able to mediate MYC cap-independent translation and is not involved in the cellular response to stress conditions.

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[P12]

## Bachmann-Bupp Syndrome – Emerging Cases of an Ultra-Rare Polyamine Disorder

EA VanSickle[1 2], J Michael[1 2], M Vipond[1 2], S Rajasekaran[1 2 3], JW Prokop[3], AS Bachmann[2 3], CP Bupp[1 2 3]

[1] *Spectrum Health, Grand Rapids MI*

[2] *International Center for Polyamine Disorders*

[3] *Michigan State University, College of Human Medicine, Grand Rapids MI*

*elizabeth.vansickle@helendevoschildrens.org*

Bachmann-Bupp Syndrome (BABS, OMIM#619075) is an ultra-rare disorder that is caused by *de novo* variants in the c-terminus of the ornithine decarboxylase 1 (*ODC1*) gene and is characterized by developmental delay, macrocephaly, alopecia, and hypotonia. *ODC1* codes for ODC, a rate-limiting enzyme in the polyamine pathway, which is essential for embryogenesis, organogenesis, and neoplastic cell growth. The mechanism for the disorder is thought to be driven by a biochemical mechanism related to abnormalities in polyamine levels, ODC protein, and enzyme activity, which is strengthened by the fact that patient gene variants continue to cluster together. To date, 9 cases of patients with BABS have been published and 3 additional patients have since been identified. The first recently published case is a 10-year-old male patient with developmental delay, hypotonia, ataxia, sparse eyelashes and eyebrows, and minimal scalp hair. He was identified to have a *de novo* heterozygous variant in the *ODC1* gene, c.1242-2A>G, (IVS11-2A>G). The second recently published case is a 6-year-old male patient with developmental delay, hypotonia, a band of hair at birth which fell out by age 1 month, sparse eyebrow hair, eyelashes, and scalp hair, and recurrent follicular cysts. Whole genome sequencing revealed a heterozygous *de novo* variant in the *ODC1* gene, c.1313_1316delCTGT (p.438Rfs*9). The third recently published case is a 23-year-old male patient with developmental delay, a history of seizures, hypotonia, and alopecia impacting the eyebrows, eyelashes, scalp, axillary, and pubic hair. Whole exome sequencing identified a heterozygous *de novo* variant in the *ODC1* gene, c.1242-2A>G (IVS11-2A>G). The fourth recently published case is a 2.5-year-old female patient with axial hypotonia, sparse hair and eyebrows, macrocephaly, and no words as of age 1.5-years-old. Trio whole exome sequencing revealed a *de novo* heterozygous variant in the *ODC1* gene, c.1252C>T (p.Gln418*). The first newly identified unpublished case is a 12-year-old female patient with moderate developmental delay, proximal myopathy, and absence of eyelashes and eyebrows. Whole exome sequencing identified a variant in the *ODC1* gene, 1242-2A>G. This variant has been reported in two other individuals with BABS, as the first recurrent variant within the condition. The second newly identified unpublished case is a 6-month-old female patient who was diagnosed shortly after birth. At birth, she presented with respiratory distress, which required intensive care hospitalization and supplemental oxygen for 4 weeks prior to being discharged still requiring supplemental oxygen. She was born with no eyelashes or eyebrows and her scalp was notable for alopecia except for a central posterior tuft of long, coarse hair. Trio whole genome sequencing revealed a *de novo* heterozygous variant in the *ODC1* gene, 1307_1311delinsT (Thr436IlefsX11). At 3.5 months of life, this patient began treatment with difluoromethylornithine (DFMO), on the same dosing strategy as published with the first BABS patient treatment. She is 4 months into treatment, currently on the intermediate DFMO dose (750 mg/m$^2$ BID), and has shown drastic clinical improvement, including hair growth, improved muscle tone, and discontinuation of supplemental oxygen., The third newly identified unpublished case is a 6-year-old male patient with global developmental delay. After performing whole exome sequencing, a stop-loss-variant, c.1385A>G, (p.Ter462Trpext*5), was identified in the *ODC1* gene. This variant has been classified as "likely pathogenic". The identification of 3 new unpublished patients with Bachmann-Bupp Syndrome brings the total number of known patients with worldwide to 12. The clinical phenotypes observed as well as the clustering pattern of variants seen in these patients

further strengthen the clinical description of this identifiable syndrome. With three of these patients now receiving treatment with DFMO, improving the understanding of this disorder will hopefully expand the option for treatment to more affected individuals and future patients who are diagnosed.

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[P13]

## Polyamines – Tissue Markers For The Differential Diagnosis And Assessment Of The Aggressiveness Of Prostate Cancer

Sophia Zaletok[1], Oleg Klenov[1], Veronika Bentrad[1], Natalia A. Ignatenko[2], Yuriy Vitruk[3], Eduard Stakhovsky[3]

[1] *R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv 03022, Ukraine*

[2] *Department of Cellular and Molecular Medicine, University of Arizona, Tucson, USA*

[3] *National Cancer Institute of the Ministry of Health of Ukraine, Kyiv 03022, Ukraine*

Prostate cancer (PCa) is one of the widespread neoplastic tumors in older men. Study of tissue markers of PCa can provide information about the proliferation and apoptosis in tumors, the ability of tumor cells to metastasize and about the mechanisms of developments of resistance to chemotherapy. Such information can facilitate the disease prognosis and development of the personalize approaches to therapy of PCa. Polyamines (PA) – spermine, spermidine putrescine – deserve a special attention as tissue markers of PCa, because of their necessity for cell proliferation. The goal of the current study was to measure the levels of polyamines and they acetyl-derivatives in the biopsy and surgical samples of benign and malignant prostate tumors for evaluation of the possibility of their use for differential diagnostic and prediction of the aggressive tumor phenotypes. The study was done using the surgical material of the PCa patients (100 cases), which differ in Gleason Score (GS) and the clinical stages of the disease (T1-TIV). Additionally, twenty samples of the patients with benign hyperplasia of the prostate (BPH), and fifteen paired normal and tumor biopsy samples of the PCa patients were analysed. The levels of PA were measured using a high pressure liquid chromatography (HPLC) system. We found that PA levels in the prostate tissue of BPH patients are significantly different from the PA levels in tumor tissue of PCa patients. Among all measured PA, the most significant difference was observed in the levels of spermine (more than 9-fold). The highest spermine levels were found in the indolent patients (GS6) and the lowest levels were observed in PCa patients with GS9 and GS10. The significant correlation was found between the decrease in spermine levels and increase in the levels of in prostate tissue GS (r=-0.86, GS6 and GS 9-10). We concluded that the significant decrease in spermine levels in PCa tumors and correlation of spermine content with the level of differentiation of PCa tumors represents an important feature of the neoplastic prostate growth and can be used as an additional marker of prostate cancer disease progression.

# AUTHORS' INDEX

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HOTEL INFORMATION
**GRAND HOTEL DUCA D'ESTE**
*Via Tiburtina Valeria, 330 – 00019 Tivoli Terme (ROMA)*

Tel: +39 0774/388552-FAX: +39 0774/388101- Green number: 800/864119

Sales Management: Federica Di Fausto

Hotel Manager: Giovanni Ricci

e-mail: f.difausto@siriohotel.com- www.siriohotel.com

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