SPANDIDOS PUBLICATIONS

WORLD ACADEMY OF SCIENCES

SAPIENZA UNIVERSITY ROME

INTERNATIONAL POLYAMINES FONDATION ONLUS

26th International Symposium on Molecular Medicine

2-4 November 2023
Grand Hotel Duca D'Este
Tivoli, Rome, Italy

Scientific Program and Abstracts

26th International Symposium on Molecular Medicine

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Scientific Program and Abstracts

SAPIENZA University of Rome
WORLD ACADEMY OF SCIENCE
Tivoli (Rome)-Italy
November 2-4, 2023

Under the auspices of

ORGANIZING COMMITTEE

Demetrios A. Spandidos , University of Crete, Greece
Enzo Agostinelli, SAPIENZA University of Rome, Italy

LOCAL COMMITTEE

E. Agostinelli
D. A. Spandidos
I. Mitsionis
R. Bordone
D. Ivy
A. Di Criscio
A. Quinto
T. Tahara
S. Tempesta

Organizing Secretariat

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**
conferences@worldacademyofsciences.com

Conference website: **https://www.polyaminesfoundation.org**
https://www.worldacademyofsciences.com/rome2023.html

Conference Information:

Open session on November 2nd at 9:00 a.m.

Welcome party on November 2nd at 8:30 p.m. at Hotel Duca D'Este

Italian dinner on November 3rd at 8:30 p.m.

Gala dinner on November 4th at 8:30 p.m.

Desk registration will be open all the time of the meeting from 8:00 a.m. to 7:00 p.m.

WELCOME MESSAGE

Dear Attendees,

The Organizing Committee has the great honor to welcome you to Italy, to attend the **26th International Symposium on Molecular Medicine** that will be held at the Grand Hotel Duca d'Este in Tivoli, Rome, Italy from November 2 to 4, 2023.

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: biochemistry, genetics, microbiology, molecular medicine, human genomics, immunology, polyamines, viral oncology and others. These topics are related to the biochemical and pathophysiological properties of polyamines, carcinogenesis and metastasis, chemotherapy, nutrition and cancer and to the traditional Chinese medicine. The scientific program will focus studies on molecular pathogenesis, molecular cardiology and psychology, on the metabolism of polyamines with a special emphasis on cancer. Findings on new pathways 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, pathological and therapeutic actions in cancer, covid and other diseases, providing a stimulus mainly for the new generation involved in the clinical field.

To enjoy stay in Tivoli-Rome to the full, you should take an active part in the social events, including parties. 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.

Demetrios A. Spandidos
University of Crete, Greece

Enzo Agostinelli
SAPIENZA
University of Rome, Italy

26th International Symposium on Molecular Medicine

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TOPICS OF THE SYMPOSIUM

1. BIOCHEMISTRY
2. CARCINOGENESIS METASTASIS
3. CHEMOTHERAPY
4. COVID
5. EPIDEMIOLOGY
6. FUNCTIONAL NUTRITION
7. GENETICS
8. HUMAN GENOMICS
9. IMMUNOLOGY
10. MICROBIOLOGY
11. MOLECULAR CARDIOLOGY
12. MOLECULAR PATHOGENESIS
13. MOLECULAR PSYCHOLOGY
14. MOLECULAR SURGERY
15. NEUROLOGY, PSYCHIATRY
16. NUTRITION AND CANCER
17. PATHOLOGY
18. POLYAMINES
19. TRADITIONAL CHINESE MEDICINE
20. TRANSGLUTAMINASES
21. VIRAL ONCOLOGY

Scientific Program

Welcome address: Professor **Enzo Agostinelli** 09:00-09:10

MOLECULAR MEDICINE/ONCOLOGY

09:10-11:00
Thursday, November 2

Chair Persons: F.S. Timeus, N. Mollo, E. Grigorieva

103. Hemophagocytic lymphohistiocytosis in clinical practice: 18 years of experience of the Onco-Hematology Department of Regina Margherita Children's Hospital. **F. Timeus**, V. Barat, N. Bertorello, E. Barisone, E. Vassallo, F. Saglio, M.L. Coniglio, E. Sieni, F. Fagioli (Italy) (25')

123. Mitochondrial dysfunction in Down syndrome is caused by an alteration in the NRIP1/PGC-1α pathway and occurs early during neuronal differentiation. **N. Mollo**, A. Limone, M. Esposito, F. Bonfiglio, C. Procaccini, A. Secondo, G. Calì, D. Sarnataro, S. Paladino, A. Conti, G. De Vita, L. Nitsch, A. Izzo (Italy) (20')

169. Glucocorticoid effects on normal brain tissue. S.D. Aladev, D.K. Sokolov, G.M. Kazanskaya, A.M. Volkov, M.O. Politko, A.V. Strokotova, S.V. Aidagulova, **E.V. Grigorieva** (Russia) (20')

153. Investigation into the validity of the MYC internal ribosome entry site and its role in resistance to stress. R. Bordone, **D.M. Ivy**, R. D'Amico, M. Barba, F. Di Pastena, B. Cesaro, A. Fatica, L. Di Magno, S. Coni, E. Agostinelli, G. Canettieri (Italy; Canada) (20')

144. Stroma-derived stimuli in FGFR2-mediated signalling in luminal breast cancer: A missing link between *in vitro* and *in vivo* findings? **H.M. Romanska**, M. Braun, J. Solek, L. Turczyk, D. Piasecka, R. Sadej (Gdansk, Poland) (15')

COFFEE/TEA BREAK 11:00-11:30

CANCER THERAPEUTICS/microRNAs

11:30-13:30
Thursday, November 2

Chair Persons: P. Vodicka, N. Tchurikov, T. Kondo

133. Genomic instability in adenomas and in colorectal cancer progression. **P. Vodička**, M. Kroupa, K. Tomasova, A. Siskova, K. Balounova, S. Vodenkova, V. Vymetalkova, R. Kumar, S. Andarawi, S. Selvi, S. Brezina, A. Gsur, K. Hemminki, L. Vodickova (Czech Republic; Austria) (25')

142. rDNA genes, development and cancer. **N.A. Tchurikov** (Russia) (25')

130. Development of novel miR-dependent genome-editing adeno-associated virus that selectively eradicates glioblastoma-initiating cells. **T. Kondo** (Japan) (25')

136. Hereditary colorectal cancer: State of the art in Lynch syndrome. **F. Duraturo**, M. De Rosa, P. Izzo (Italy) (25')

165. CyTOF and microRNAs reveal possible biomarkers for early diagnosis and treatment of heparin induced thrombocytopenia (HIT). E. Kourepini, **G. Soufla**, E. Iliopoulou, S. Georgantis, T. Kanellopoulou, D. Boumpas, T. Kostelidou (Greece) (20')

LUNCH 13:30 - 14:30

MOLECULAR MEDICINE/WOUND HEALING

15:00-17:00
Thursday, November 2

Chair Persons: E. Priel, P. Nielsen, P. Gal

117. The beneficial effects of novel telomerase increasing compounds on neurodegenerative and ageing related diseases. **E. Priel** (Israel) (20')

112. New weapons against multidrug resistant bacterial infections via an RNA therapeutics antibiotic drug discovery platform. **P. Nielsen** (Denmark) (20')

114. Novel approaches to treat wounds. **P. Gál**, T. Vasilenko, D. Lukáš, R. Zajíček, D. Rejman (Slovak Republic; Czech Republic) (20')

113. The sweet side of wound healing: galectins as promising therapeutic targets in the treat-ment of open excisions and sutured incisions. **V. Gálová**, T. Vasilenko, L. Urban, K. Smetana Jr, H. Kaltner, P. Gál (Slovak Republic; Czech Republic; Germany) (20')

115. Agrimonia eupatoria L. and skin wound healing. **T. Vasilenko**, I. Kováč, A. Vrzgula, K. Smetana Jr, P. Gál (Slovak Republic; Czech Republic) (20')

121. Canonical and non-canonical transforming factor-beta signaling in fibroblasts isolated from various neoplastic/healing/ normal tissues. **M. Coma** (Slovakia) (20')

COFFEE/TEA BREAK 17:00-17:30

POLYAMINES

17:30-19:30
Thursday, November 2

Chair Persons: E. Agostinelli, T. Oka, A. Kaiser

104. The role of spermidine and its key metabolites in important, pathogenic human viruses and in parasitic infections caused by *Plasmodium*. **A. Kaiser** (Germany) (30')

126. Polyamine/EIF5A axis in colorectal tumors. S. Coni, **R. Bordone**, D.M. Ivy, Z.N. Yurtsever, L. Di Magno, R. D'Amico, B. Cesaro, A. Fatica, F. Belardinilli, F. Bufalieri, M. Maroder, E. De Smaele, L. Di Marcotullio, G. Giannini, E. Agostinelli, G. Canettieri (Italy) (20')

105. Dose-dependent effect of polyamines on cancer cell proliferation and viability. **T. Tahara**, L. Di Magno, S. Coni, R. Bordone, D. Ivy, L. Rutigliano, R. Pellegrino, A. Montella, G. Canettieri, E. Agostinelli (Italy) (20')

141. Exploring novel ligands as potential inhibitors and substrates for bovine serum amine oxidase: Biochemical insights and molecular modeling studies. R. Ragno, **E. Proia**, A. Ragno, E. Agostinelli (Italy) (20')

111. C-Methylated analogues of spermidine: Synthesis and biological application. A.I. Salikhov, **M.A. Khomutov** (Russia) (15')

152. Absorption and effects of SAMN@TA@BSAO nanohybrid on Caco-2 cells. **F. Tonolo**, L. Rutigliano, G. Rilievo, M. Magro, M.L. Di Paolo, M.P. Rigobello, E. Agostinelli, F. Vianello (Italy) (15')

WELCOME DINNER 20:30
Grand Hotel Duca D'Este

BIOCHEMISTRY/MOLECULAR MEDICINE

09:00-11:00
Friday, November 3

Chair Persons: R. Gambari, A. Finotti

106. Expression of γ-globin genes in sirolimus-treated β-thalassemia patients. C. Zuccato, L.C. Cosenza, M. Zurlo, A. Finotti, I. Lampronti, M. Borgatti, C. Scapoli, M. Prosdocimi, M.R. Gamberini, **R. Gambari** (Italy) (25')

108. The cystic transmembrane conductance regulator gene (CFTR) is under post-transcriptional control of microRNAs: Effects of treatments with ago-miRNAs and possible biomedical applications. **C. Papi**, J. Gasparello, M. Zurlo, R. Gambari, A. Finotti (Italy) (25')

124. Gene editing for the therapy of β-thalassemia: Combining CRISPR-Cas9-based gene correc-tion and fetal hemoglobin induction. L.C. Cosenza, C. Zuccato C, M. Zurlo, R. Gambari, **A. Finotti** (Italy) (25')

168. Involvement of autophagy as detoxifying mechanism in erythroid cells of β-thalassemia patients accumulating free α-globin. **M. Zurlo**, C. Zuccato, L.C. Cosenza, C. Papi, G. Breveglieri, A. Finotti, R. Gambari (Italy) (25')

116. LncRNA MALAT1, post-transcriptionally stabilized by NSUN2-mediated M5C modification, exerts properties in bone lesions formation in multiple myeloma. **X. Cui**, M. Yu (P.R. China) (20')

COFFEE/TEA BREAK 11:00-11:30

PHARMACOLOGY/MOLECULAR MEDICINE

11:30-13:10
Friday, November 3

Chair Persons: R. Reiter, K. Mio, M. Jirásko

101. Melatonin and its metabolites: Major contributors to the maintenance of cellular redox homeostasis. **R. Reiter** (USA) (40')

172. Real-time observation of capsaicin-induced intramolecular domain dynamics of TRPV1 using the diffracted X-ray tracking method. **K. Mio**, T. Ohkubo, D. Sasaki, T. Arai, M. Sugiura, S. Fujimura, Y.C. Sasaki (Japan) (20')

138. Assessment of preanalytical conditions of prostate markers. **M. Jirásko**, R. Kučera, R. Vrzáková, V. Šimánek, R. Viták, O. Topolčan (Czech Republic) (20')

119. Autophagy promotes insulin secretion under glucose stimulation both *in vitro* and *in vivo* in a RAB37 dependent manner. S.-Y. Wu, Y.-C. Wang, S.-H. Lan, **H.-S. Liu** (Taiwan) (20')

122. Effects of high intensity interval vs. low intensity continuous training on LXRβ, ABCG5 and ABCG8 genes expression in male Wister rats. **S. Jalali**, M. Jafari (Tehran; Shirvan, Iran) (20')

LUNCH 13:30 - 14:30

MOLECULAR ONCOLOGY

15:00-17:00
Friday, November 3

Chair Persons: A. El-Naggar, A. Semczuk, A.R. Khomutov

102. Inter and intra-tumoral heterogeneity of salivary adenoid cystic carcinoma: Implications for stratification and targeted therapy. **A. El-Naggar** (USA) (40')

135. HPV16 E6 gene transcripts in primary type II endometrial carcinomas. W. Szewczuk, O. Szewczuk, K. Czajkowski, M. Wałędziak, B. Górnicka, T. Ilczuk, W. Kawecka, **A. Semczuk** (Poland) (30')

118. Phosphorus analogues of glutamic acid and S-adenosylmethionine: Synthesis and biological activity. **A.R. Khomutov**, F. Giovannercole, M.V. Demiankova, V.L. Filonov, M.A. Khomutov, O.V. Efremenkova, D. De Biase (Moscow, Russia; Latina, Italy) (20')

129. The prognostic value of LAYN in HPV-related head and neck squamous cell carcinoma and its influence on immune cell infiltration. **C. Zhu** (P.R. China) (20')

134. H3K27me3-mediated inactivation of SFRP1 promotes cell proliferation via Wnt/β-Catenin signaling pathway in esophageal squamous cell carcinoma. M. Zhou, S. Yu, Y. Xu, M. Liu, Y. Ge, **H. Fan** (P.R. China) (10')

COFFEE/TEA BREAK 17:00-17:30

MOLECULAR BIOLOGY/ONCOLOGY

17:30-19:30
Friday, November 3

Chair Persons: D. Batchvarov, J.D. Schuetz, H. Rokita

151. Aberrant DNA methylation potentiates oncogenes' expression and disease progression in ovarian cancer. **D. Batchvarov** (Canada) (25')

159. Tumor-acquired somatic mutation disrupts the TMD-NBD communication and transport. T. Gose, A. Rasouli, S. Dehghani-Ghahnaviyeh, P.-C. Wen, Y. Wang, J. Lynch, Y. Fukuda, T. Shafi, R.C. Ford, E. Tajkhorshid, **J.D. Schuetz** (Memphis, TN; Urbana, IL, USA; Manchester, UK) (25')

161. Effects of silencing of the *PHLDA1* gene on gene expression and metabolism of human neuroblastoma cells. B. Bugara, M. Durbas, M. Kudrycka, A. Malinowska, I. Horwacik, **H. Rokita** (Poland) (25')

127. Research and analysis on CT signs and clinical characteristics of chronic duodenal papilla mucositis and duodenal papillary carcinoma. **N. Wang** (P.R. China) (25')

Dental pulp MSCs from osteopetrosis patients treated with the carbonic anhydrase activator- azole stimulate osteogenic and immune metabolic pathways. **A. Ayala** (Saudi Arabia) (20')

ITALIAN DINNER 20:30
Grand Hotel Duca D'Este

EXPERIMENTAL THERAPEUTICS

09:00-11:00
Saturday, November 4

Chair Persons: Y. Suzuki-Karasaki, I. Horwacik, W. Zhu

131. Changes in mitochondrial morphology and positioning in cancer cell death caused by mitochondrial oxidative stress. **Y. Suzuki-Karasaki**, M. Suzuki-Karasaki (Japan) (25')

120. Aqueous ozone exhibits anticancer activity by triggering mitochondria-targeted oxidative cell death. **M. Suzuki-Karasaki**, Y. Ochiai, S. Innami, H. Okajima, M. Suzuki-Karasaki, H. Nakayama, Y. Suzuki-Karasaki (Japan) (25')

160. Investigation of mechanisms of direct cytotoxicity of anti-GD2 ganglioside monoclonal anti-bodies using neuroblastoma cell cultures. **I. Horwacik**, H. Rokita (Poland) (25')

154. Endogenous morphine in mollusk and human: Neuro-immune modulation. **W. Zhu** (USA) (25')

155. Investigating the potential of plant-derived antimalarial drugs, Artemisinin and Artesunate, in alleviating long COVID symptoms. A. Qian, **W. Zhu** (USA) (20')

COFFEE/TEA BREAK 11:00-11:30

MOLECULAR/CLINICAL ONCOLOGY/GENOME INSTABILITY

11:30-13:30
Saturday, November 4

Chair Persons: L. Vannucci, L. Falzone

162. Smoldering inflammation sustains tissue structure changes and tumor microenvironment development. **L. Vannucci**, F. Čaja, D. Stakheev, P. Lukác, L. Rajsiglova, P. Tenti, D. Vondrasek, G. Mucciolo, R. Štěpánková, P. Makovický, P. Makovický, T. Hudcovic, P. Šima, D. Smrz (Czech Republic; Slovakia) (25')

170. Molecular determinants of drug resistance in colorectal cancer organoids and novel integrated treatment approaches. **L. Falzone**, G. Spoto, D. Ricci, S. Candido, M. Libra (Italy) (25')

171. Novel insights into the epigenetic regulation of SLC22A17 in cutaneous melanoma. Validation of the cg17199325 methylation hotspot as potential diagnostic biomarker. **A. Lavoro**, L. Falzone, G. Gattuso, G.N. Conti, M. Libra, S. Candido (Italy) (25')

167. Oncogenic potential of AAA+ ATPase proteins in cancer. **A. Nayak** (India) (25')

Closing talk: The cancer story and publishing in biomedical sciences. **D.A. Spandidos** (Greece) (20')

EXCURSION TO TIVOLI 14:30-19:30

GALA DINNER 20:30
Grand Hotel Duca D'Este

POSTERS (Poster viewing during the entire symposium)

107. Inhibitory effects of SARS-CoV-2 Spike protein and BNT162b2 vaccine on erythropoietin-induced globin gene expression in erythroid precursor cells (ErPCs) from β-thalassemia patients. L.C. Cosenza, G. Marzaro, M. Zurlo, J. Gasparello, C. Zuccato, A. Finotti, **R. Gambari** (Italy)

109. Inhibition of the expression of pro-inflammatory genes by pre-miR-93-5p: Relevance for cystic fibrosis and COVID-19. J. Gasparello, M. Zurlo, C. Papi, **A. Finotti**, E. Agostinelli, R. Gambari (Italy)

110. Antiviral activity of AGE and SAC against different viruses: preliminary study. C. Nonne, R. Campagna, D.E. Compagnino, M.G. Leone, G. Sfara, G. Canettieri, O. Turriziani, **E. Agostinelli** (Italy)

125. Novel stevioside derivative induced apoptosis in colon cancers cells via mitochondrial signaling pathways. **A. Malki**, A. El Sharkawy (Qatar; Italy)

128. Therapeutic efficacy of sorafenib and plant-derived phytochemicals in human colorectal cancer cells. **M.-S. Abaza I**, A.-M. Bahman, S. Khoushaish and R.J. Al-Attiyah (Kuwait)

132. From liquid biopsy to microRNA therapeutics for colorectal cancer (CRC). J. Gasparello, M. Zurlo, C. Papi, P. Giacomini, R. Corradini, R. Gambari, **A. Finotti** (Ferrara; Rome; Parma, Italy)

137. METTL18 is a novel phenotypic regulator of Src-dependent metastatic response of HER2-negative breast cancer. H.G. Kim, J.H. Kim, **J.Y. Cho** (Republic of Korea)

139. MicroRNA-204 inhibits vasculogenic mimicry and angiogenesis in stem-like breast cancer cells. M. Resendiz-Hernández, E. Contreras-Sanzón, M.B. Silva-Cázares, L.A. Marchat, O.N.H. De la Cruz, **M.C. López-Camarillo** (Mexico)

140. Proteoglycans and glycosaminoglycans contribute to high heterogeneity and chemoresistance of glioblastoma cells. S.A. Nikitina, D.K. Sokolov, A.Y. Tsidulko, A.V. Strokotova, E. Fasler-Kan, **E.V. Grigorieva** (Russia; Switzerland)

143. Comparison of specific myo/fibroblast markers in fibroblasts isolated from various neoplastic/healing/normal tissues. **L. Urban** (Slovak Republic)

145. CRISPR/Cas9 screens to define the membrane transporters at the metabolic intersection with hypusination of eIF5A. **Y.K. Law**, P. Essletzbichler, G. Superti-Furga (Austria)

146. A new mechanism of oxidative DNA damage by a putative acrylamide metabolite, acrylohydroxamic acid. **Y. Mori**, H. Kobayashi, M. Murata, S. Oikawa (Japan)

147. Development of a rapid diagnostic test for detection of pathogenic mycobacteria based on CRISPR. **L. Lima**, M. Quinhões, S. Vasconcellos, R. Teixeira, E. Machado, B. Petrilho, V. Moraes, C. Cristhine, H. Gomes, P. Suffys (Brazil)

148. Antineoplastic and antiangiogenic effects of novel tyrosine kinase inhibitors in hepatocel-lular cancer cells. A. Ma, N. Göhringer, B. Nitzsche, B. Biersack, **M. Höpfner** (Germany)

149. Tumor microenvironment-dependent epigenetic imprinting in the vasculature predicts colon cancer outcome. E. Naschberger, M. Fuchs, N. Dickel, M. Kunz, B. Popp, C.G. Anchang, R. Demmler, Y. Lyu, S. Uebe, A.B. Ekici, C.I. Geppert, A. Hartmann, C. Flierl, K. Petter, T. Gass, S. Völkl, M. Scharl, A. Ramming, C. Günther, S. Merkel, V.S. Schellerer, **M. Stürzl** (Germany; Switzerland)

150. Aged garlic extract and S-allyl cysteine reduce the content of pro-inflammatory mRNAs in bronchial epithelial IB3-1 cells treated with SARS-CoV-2 Spike protein and BNT162b2 vaccine. J. Gasparello, C. Papi, G. Breveglieri, M. Zurlo, A. Finotti, **R. Gambari**, E. Agostinelli (Italy)

156. Chlorpyrifos: Effects on cell cytotoxicity, viability, and apoptosis via mitochondrial complex I and caspase III. S. Chen, **W. Zhu** (USA)

157. Aloin induces cytotoxicity and generates cell death in lymphoma cells through the TNF pathway. E. Huang, **W. Zhu** (USA)

158. Discovering apoptotic drug targets for colorectal cancer through innovative soft voting machine learning approach. Y. Zuo, **W. Zhu** (USA)

163. Iron oxide-based nanohybrids as universal carriers for cellular delivery. **G. Rilievo**, F. Tonolo, L. Rutigliano, M. Magro, M.L. Di Paolo, M. Cervelli, A. Ilari, A. Liuzzi, E. Agostinelli, F. Vianello (Italy)

164. Cytotoxic effects of magnetic nanohybrids on colorectal adenocarcinoma cell models. **L. Rutigliano**, F. Tonolo, G. Rilievo, M. Magro, M.L. Di Paolo, M.P. Rigobello, G. Canettieri, E. Agostinelli, F. Vianello (Italy)

166. Combined treatment with pre-miR-93 and products from *Allium sativum* reduces interleu-kin-8 mRNA content induced by anti-SARS-CoV-2 BNT162b2 vaccine in bronchial epithe-lial IB3-1 cells. **C. Papi**, J. Gasparello, G. Breveglieri, M. Zurlo, A. Finotti, R. Gambari, E. Agostinelli (Italy)

26th International Symposium on Molecular Medicine
November 2nd to 4th, 2023 Tivoli (Rome), Italy.
Conference Registration List

MOHAMED **ABAZA** MOLECULAR BIOLOGY PROGRAM, DEPARTMENT OF BIOLOGICAL SCIENCES, FACULTY OF SCIENCE, KUWAIT UNIVERSITY, P.O. BOX 5969, SAFAT 13060, KUWAIT PHONE: 50967733 EMAIL: MOHAMMED.ABAZA@KU.EDU.KW **POSTER PRESENTATION**	ENZO **AGOSTINELLI** DEPARTMENT OF SENSORY ORGANS, FACULTY MEDICINE AND DENTISTRY, SAPIENZA UNIVERSITY OF ROME, UNIVERSITY HOSPITAL POLICLINICO UMBERTO I, VIALE DEL POLICLINICO 155, I-00161 ROME, ITALY PHONE: + 39(0)649976857 EMAIL: ENZO.AGOSTINELLI@UNIROMA1.IT **ORGANIZER**
AYODELE **ALAIYA** 1 TAKASSOUSI RIYADH, RIYADH 11211 SAUDI ARABIA PHONE: +966551138605 EMAIL: AALAIYA@KFSHRC.EDU.SA **SPEAKER**	DIMTCHO **BATCHVAROV** DÉPARTEMENT DE MÉDECINE MOLECULAIRE, UNIVERSITÉ LAVAL, CRCHU-Q, CENTRE DE RECHERCHE EN CANCÉROLOGIE QUÉBEC PQ, CANADA PHONE: 5818880223 EMAIL: DIMTCHO.BATCHVAROV@CRCHU DEQUEBEC.ULAVAL.CA **SPEAKER**
ROSA **BORDONE** DEPARTMENT OF MOLECULAR MEDICINE, UNIVERSITY OF ROME "SAPIENZA", VIALE REGINA ELENA, 00161, ROME, ITALY MAIL: ROSA.BORDONE@UNIROMA1.IT **SPEAKER**	XIAOCHUN **CAI** HUANGPU DISTRICT SCIENCE CITY INTERNATIONAL ENTERPRISE INCUBATOR D BUILDING 201-203 GUANGZHOU, GUANGDONG PROVINCE 510700 CHINA PHONE: 13760696178 MAIL: CAIXIAOCHUN@YUJIAB.COM **ATTENDEE**

GIANLUCA **CANETTIERI**

DEPARTMENT OF MOLECULAR MEDICINE, UNIVERSITY OF ROME "SAPIENZA", VIALE REGINA ELENA, 00161, ROME, ITALY

EMAIL: GIANLUCA.CANETTIERI@UNNIROMA1.IT

ATTENDEE

JAE YOUL **CHO**

DEPARTMENT OF INTEGRATIVE BIOTECHNOLOGY, SUNGKYUNKWAN UNIVERSITY, SUWON 16419, REPUBLIC OF KOREA

PHONE: +821098520046
E-MAIL: JAECHO@SKKU.EDU

POSTER PRESENTATION

MATUS **COMA**

DEPARTMENT OF PHARMACOLOGY, FACULTY OF MEDICINE, UNIVERSITY OF PAVOL JOZEF SAFARIK IN KOSICE, KOSICE.

PHONE: +421918114256
E-MAIL: COMA.MATUS@GMAIL.COM

POSTER PRESENTATION

ANNAMARIA **DI CRISCIO**

DEPARTMENT OF SENSORY ORGANS, FACULTY MEDICINE AND DENTISTRY, SAPIENZA UNIVERSITY OF ROME, UNIVERSITY HOSPITAL POLICLINICO UMBERTO I, VIALE DEL POLICLINICO 155, I-00161 ROME, ITALY

PHONE: 3313547224
EMAIL: DICRISCIO.1838452@STUDENTI.UNIROMA1.IT

TEAM ORGANIZATION

ADEL K. **EL-NAGGAR**

M.D. ANDERSON CANCER, HUSTON, TX, USA

EMAIL: ANAGGAR@MDANDERSON.ORG

SPEAKER

ALESSIA **FINOTTI**

CENTER CHIARA GEMMO AND ELIO ZAGO FOR THE RESAERCH ON THALASSEMIA, UNIVERSITY OF FERRARA, FERRARA, ITALY

PHONE: 0532974510
E-MAIL: ALESSIA.FINOTTI@UNIFE.IT

SPEAKER

PETER **GÁL**

DEPARTMENT OF BIOMEDICAL RESEARCH, EAST-SLOVAK INSTITUTE OF CARDIOVASCULAR DISEASES, INC., KOŠICE, SLOVAK REPUBLIC.

PHONE +421905236876
EMAIL GALOVCI@YAHOO.COM

SPEAKER

VLASTA **GÁLOVÁ**

DEPARTMENT OF BIOMEDICAL RESEARCH, EAST-SLOVAK INSTITUTE OF CARDIOVASCULAR DISEASES, INC., KOŠICE, SLOVAK REPUBLIC.

PHONE +421907620838
EMAIL VLASTA.PERZELOVA@GMAIL.COM

SPEAKER

ROBERTO **GAMBARI** DEPARTMENT OF LIFE SCIENCES AND BIOTECHNOLOGY, UNIVERSITY OF FERRARA, FERRARA, ITALY EMAIL: GAM@UNIFE.IT PHONE: 0532974443 **SPEAKER**	LIA LIMA **GOMEZ** LABORATORY OF MOLECULAR BIOLOGY APPLIED TO MYCOBACTERIA -FIOCRUZ. BRAZIL PHONE: 5521979654624 E-MAIL: LIALIMAGOMES@HOTMAIL.COM **POSTER PRESENTATION**
ELVIRA V. **GRIGORIEVA** INSTITUTE OF MOLECULAR BIOLOGY AND BIOPHYSICS FRC FTM E-MAIL: ELV_GRIG@YAHOO.COM **SPEAKER**	MICHAEL **HOPFENER** INSTITUTE OF PHYSIOLOGY, CHARITÉ-UNIVERSITÄTSMEDIZIN BERLIN, CORPORATE MEMBER OF THE FREIE UNIVERSITÄT BERLIN, HUMBOLDTUNIVERSITÄT ZU BERLIN AND BERLIN INSTITUTE OF HEALTH, CHARITÉPLATZ 1, 10117 BERLIN, GERMANY PHONE: +4930450528515 E-MAIL: MICHAEL.HOEPFNER@CHARITE.DE **POSTER PRESENTATION**
IRENA **HORWACIK** LABORATORY OF MOLECULAR GENETICS AND VIROLOGY, FACULTY OF BIOCHEMISTRY, BIOPHYSICS AND BIOTECHNOLOGY, JAGIELLONIAN UNIVERSITY, GRONOSTAJOWA 7 ST., KRAKÓW, POLAND PHONE: 48126646369 E-MAIL: IRENA.HORWACIK@UJ.EDU.PL **SPEAKER**	DEVON MICHAEL **IVY** DEPARTMENT OF MOLECULAR MEDICINE, UNIVERSITY OF ROME "SAPIENZA", VIALE REGINA ELENA, 00161, ROME, ITALY PHONE: +393298293537 EMAIL: DEVON.IVY@UNIROMA1.IT **SPEAKER**

MICHAL **JIRÁSKO**

DEPARTMENT OF IMMUNOCHEMISTRY DIAGNOSTICS, UNIVERSITY HOSPITAL AND FACULTY OF MEDICINE IN PILSEN; DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, FACULTY OF MEDICINE IN PILSEN, CHARLES UNIVERSITY, CZECH REPUBLIC

PHONE: +420608913706
MAIL: JIRASKOM@FNPLZEN.CZ

SPEAKER

ANNETTE **KAISER**

MEDICAL RESEARCH CENTRE, UNIVERSITY OF DUISBURG-ESSEN, HUFELANDSTRASSE 55, 45147 ESSEN, GERMAN

EMAIL: KAISER@MICROBIOLOGY-BONN.DE
PHONE: 491738926333

SPEAKER

ALEX **KHOMUTOV**

ENGELHARDT INSTITUTE OF MOLECULAR BIOLOGY, RUSSIAN ACADEMY OF SCIENCES, MOSCOW, RUSSIA

EMAIL: ALEXKHOM@LIST.RU

SPEAKER

MAXIM **KHOMUTOV**

ENGELHARDT INSTITUTE OF MOLECULAR BIOLOGY, RUSSIAN ACADEMY OF SCIENCES, MOSCOW, RUSSIA

PHONE: 84991356065
EMAIL: MAKHOMUTOV@MAIL.RU

SPEAKER

TORU **KONDO**

DIVISION OF STEM CELL BIOLOGY, INSTITUTE FOR GENETIC MEDICINE, HOKKAIDO UNIVERSITY, KITA-15, NISHI-7, KITA-KU SAPPORO, HOKKAIDO 060-081 JAPAN

PHONE 0117066082
EMAIL TKONDO@IGM.HOKUDAI.AC.JP

SPEAKER

YEE KWAN **LAW**

CEMM RESEARCH CENTRE FOR MOLECULAR MEDICINE OF THE AUSTRIAN ACADEMY OF SCIENCES, VIENNA AUSTRIA

PHONE: 06609250835
EMAIL: YLAW@CEMM.OEAW.AC.AT

POSTER PRESENTATION

MARIO CESAR **LOPEZ CAMARILLO**

POSGRADO EN CIENCIAS GENÓMICAS, UNIVERSIDAD AUTÓNOMA DE LA CIUDAD DE MÉXICO, MÉXICO

PHONE: 5547268128
EMAIL: CESAR.LOPEZ@UACM.EDU.MX

POSTER PRESENTATION

AHMED **MALKI**

DEPARTMENT OF BIOMEDICAL SCIENCES, COLLEGE OF HEALTH SCIENCES, QU HEALTH, QATAR UNIVERSITY, DOHA, QATAR

PHONE: +97430077982
E-MAIL: AHMED.MALKI@QU.EDU.QA

POSTER PRESENTATION

KAZUHIRO **MIO** OPERANDO-OIL, NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY JAPAN PHONE**:** +81335998104 E-MAIL: KAZU.MIO@AIST.GO.JP **SPEAKER**	IOANNIS **MITSIONIS** SPANDIDOS PUBLICATIONS, ATHENS, GREECE AND LONDON, UK EMAIL: MITSIONIS@SPANDIDOSPUBLICATIONS.COM **TEAM ORGANIZATION**
YURIE **MORI** hDEPARTMENT OF ENVIRONMENTAL AND MOLECULAR MEDICINE, MIE UNIVERSITY GRADUATE SCHOOL OF MEDICINE, EDOBASHI 2-174, TSU, MIE, 514-8507, JAPAN PHONE: +81592315011 E-MAIL: M-YURIE@MED.MIE-U.AC.JP **POSTER PRESENTATION**	MARIKO **MURATA** DEPARTMENT OF ENVIRONMENTAL AND MOLECULAR MEDICINE, MIE UNIVERSITY GRADUATE SCHOOL OF MEDICINE, EDOBASHI 2-174, TSU, MIE, 514-8507, JAPAN PHONE: +81592315011 E-MAIL: MMURATA@MED.MIE-U.AC.JP **POSTER PRESENTATION**
HIROO **MURATA** 2-1-1,TSUDAHIGASHIMACHI HIRAKATA, OSAKA 5730122 JAPAN PHONE: 0728589323 E-MAIL: M-UEDA@HIARAKATARYOIKUE-MED.OR.JP **ATTENDEE**	ADITI **NAYAK** DEPARTMENT OF LIFE SCIENCE, GURU NANAK INSTITUTE OF PHARMACEUTICAL SCIENCE AND TECHNOLOGY, 157/F, NILGUNJ RD, SAHID COLONY, PANIHATI, KOLKATA, WEST BENGAL-700114, INDIA PHONE: 917978626436 EMAIL: ADITI.NAYAK@GNIPST.AC.IN **POSTER PRESENTATION SPEAKER**

ELISABETH **NASCHBERGER** DIVISION OF MOLECULAR AND EXPERIMENTAL SURGERY, TRANSLATIONAL RESEARCH CENTER, UNIVERSITÄTSKLINIKUM ERLANGEN, ERLANGEN-NÜRNBERG (FAU), ERLANGEN, 91054, GERMANY AND COMPREHENSIVE CANCER CENTER ERLANGEN-EMN, ERLANGEN, 91054 PHONE: 015170160548 **POSTER PRESENTATION**	PETER **NIELSEN** CENTER FOR PEPTIDE-BASED ANTIBIOTICS, DEPARTMENT OF CELLULAR AND MOLECULAR MEDICINE, FACULTY OF HEALTH AND MEDICAL SCIENCES, UNIVERSITY OF COPENHAGEN, DENMARK PHONE : 25248309 EMAIL : PTRN@SUND.KU.DK **SPEAKER**
CHIARA **NONNE** DEPARTMENT OF MOLECULAR MEDICINE, UNIVERSITY OF ROME "SAPIENZA", VIALE REGINA ELENA, 00161, ROME, ITALY EMAIL : CHIARANONNE@UNIROMA1.IT **SPEAKER**	TAKAHIRO **OGAWA** WAKUNAGA PHARMACEUTICAL CO. LTD HIROSHIMA, JAPAN EMAIL: WAKUNAGA_H@WAKUNAGA.CO.JP **ATTENDEE**
SHINJI **OIKAWA** DEPARTMENT OF ENVIRONMENTAL AND MOLECULAR MEDICINE, MIE UNIVERSITY GRADUATE SCHOOL OF MEDICINE, EDOBASHI 2-174, TSU, MIE, 514-8507, JAPAN PHONE: +81592315011 MAIL: S-OIKAWA@MED.MIE-U.AC.JP **POSTER PRESENTATION**	TAKAMI **OKA** WAKUNAGA PHARMACEUTICAL CO. LTD HIROSHIMA, JAPAN EMAIL: WAKUNAGA_H@WAKUNAGA.CO.JP **ATTENDEE**
YUKIE **OKAZAKI** 2-1-1,TSUDAHIGASHIMACHI HIRAKATA, OSAKA 5730122 JAPAN PHONE: 0728589323 EMAIL: MASAKI@HIRAKATARYOIKU-MED.OR.JP **ATTENDEE**	CHIARA **PAPI** DEPARTMENT OF LIFE SCIENCES AND BIOTECHNOLOGY, UNIVERSITY OF FERRARA, FERRARA, ITALY EMAIL: CHIARA.PAPI@UNIFE.IT **SPEAKER**

ELEONORA **PROIA** ROME CENTER FOR MOLECULAR DESIGN, DEPARTMENT OF DRUG CHEMISTRY AND TECHNOLOGY, SAPIENZA UNIVERSITA' DI ROMA, P. le A. Moro 5, 00185 ROMA, ITALY. EMAIL: ELEONORA.PROIA@UNIROMA1.IT **SPEAKER**	ALESSIA **QUINTO** DEPARTMENT OF SENSORY ORGANS, FACULTY MEDICINE AND DENTISTRY, SAPIENZA UNIVERSITY OF ROME, UNIVERSITY HOSPITAL POLICLINICO UMBERTO I, VIALE DEL POLICLINICO 155, I-00161 ROME, ITALY EMAIL: QUINTO.1749578@STUDENTI.UNIROMA1.IT PHONE: 3286265339 **TEAM ORGANIZATION**
RUSSEL J **REITER** UT HEALTH SAN ANTONIO, LONG SCHOOL OF MEDICINE, SAN ANTONIO, TX, USA EMAIL: REITER@UTHSCSA.EDU **SPEAKER**	GRAZIANO **RILIEVO** DEPARTMENT OF COMPARATIVE BIOMEDICINE AND FOOD SCIENCE, UNIVERSITY OF PADUA, VIALE DELL'UNIVERSITÀ 16, 35020, LEGNARO (PD), ITALY PHONE. 3465920409 EMAIL: GRAZIANO.RILIEVO@PHD.UNIPD.IT **POSTER PRESENTATION**
RINO **RAGNO** ROME CENTER FOR MOLECULAR DESIGN, DEPARTMENT OF DRUG CHEMISTRY AND TECHNOLOGY, SAPIENZA UNIVERSITA' DI ROMA, P. le A. Moro 5, 00185 ROMA, ITALY. EMAIL: RINO.RAGNO@UNIROMA1.IT **ATTENDEE**	HANNA **ROKITA** LABORATORY OF MOLECULAR GENETICS AND VIROLOGY, FACULTY OF BIOCHEMISTRY, BIOPHYSICS AND BIOTECHNOLOGY, JAGIELLONIAN UNIVERSITY, KRAKOW, POLAND PHONE : 48126646337 EMAIL : HANNA.ROKITA@UJ.EDU.PL **SPEAKER**

HANNA **ROMANSKA** DEPARTMENT OF PATHOLOGY, MEDICAL UNIVERSITY OF LODZ PHONE: 506633338 EMAIL: HANNA.ROMANSKA@GMAIL.COM **SPEAKER**	LAVINIA **RUTIGLIANO** DEPARTMENT OF SENSORY ORGANS, FACULTY MEDICINE AND DENTISTRY, SAPIENZA UNIVERSITY OF ROME, UNIVERSITY HOSPITAL POLICLINICO UMBERTO I, VIALE DEL POLICLINICO 155, I-00161 ROME, ITALY PHONE:3463155723 EMAIL: LAVINIA.RUTIGLIANO@UNIROMA1.IT **POSTER PRESENTATION**
AKANE **SAITO** 2-1-1,TSUDAHIGASHIMACHI HIRAKATA, OSAKA 5730122 JAPAN PHONE: 0728589323 EMAIL: A.SAITO@HIRAKATARYOIKU-MED.OR.JP **ATTENDEE**	TOMOMI **SATO** 2-1-1,TSUDAHIGASHIMACHI HIRAKATA, OSAKA 5730122 JAPAN PHONE: 0728589323 EMAIL: BA80393S_S@YAHOO.CO.JP **ATTENDEE**
DEMETRIOS A. **SPANDIDOS** MEDICAL, SCHOOL, UNIVERSITY OF CRETE, HERAKLION, GREECE EMAIL: SPANDIDOS@SPANDIDOS.GR **ORGANIZER**	MICHAEL **STURZL** DIVISION OF MOLECULAR AND EXPERIMENTAL SURGERY, TRANSLATIONAL RESEARCH CENTER, UNIVERSITÄTSKLINIKUM ERLANGEN, ERLANGEN-NÜRNBERG (FAU), ERLANGEN, 91054, GERMANY AND COMPREHENSIVE CANCER CENTER ERLANGEN-EMN, ERLANGEN, 91054 PHONE : 015170160548 E-MAIL : MICHAEL.STUERZL@UK-vERLANGEN.DE **POSTER PRESENTATION**

MANAMI **SUZUKI-KARASAKI** PLASMA CHEMIBIO LABORATORY, NASUSHIOBARA, TOCHIGI, JAPAN. GRADUATE SCHOOL OF MEDICAL SCIENCES, KUMAMOTO UNIVERSITY, KUMAMOTO, JAPAN. PHONE: E-MAIL: KSMANAMI181@GMAIL.COM **SPEAKER**	YOSHIHIRO **SUZUKI-KARASAKI** PLASMA CHEMIBIO LABORATORY, NASUSHIOBARA, TOCHIGI, JAPAN GRADUATE SCHOOL OF MEDICAL SCIENCES, KUMAMOTO UNIVERSITY, KUMAMOTO, JAPAN. PHONE: E-MAIL: SUZUKI.PCBL@GMAIL.COM **SPEAKER**
TOMOAKI **TAHARA** DEPARTMENT OF SENSORY ORGANS, FACULTY MEDICINE AND DENTISTRY, SAPIENZA UNIVERSITY OF ROME, UNIVERSITY HOSPITAL POLICLINICO UMBERTO I, VIALE DEL POLICLINICO 155, I-00161 ROME, ITALY EMAIL: TAHARA.1943656@STUDENTI.UNIROMA1.IT **SPEAKER**	NICKOLAI A. **TCHURIKOV** ENGELHARDT INSTITUTE OF MOLECULAR BIOLOGY RUSSIAN ACADEMY OF SCIENCES, MOSCOW, RUSSIA PHONE: +74991359753 EMAIL:TCHURIKOV@EIMB.RU **SPEAKER**
SILVIA **TEMPESTA** DEPARTMENT OF SENSORY ORGANS, FACULTY MEDICINE AND DENTISTRY, SAPIENZA UNIVERSITY OF ROME, UNIVERSITY HOSPITAL POLICLINICO UMBERTO I, VIALE DEL POLICLINICO 155, I-00161 ROME, ITALY EMAIL: TEMPESTA.1898863@STUDENTI.UNIROMA1.IT TELEFONO:3292094420 **TEAM ORGANIZATION**	FABIO STEFANO **TIMEUS** PEDIATRIC ONCO-HEMATOLOGY DEPARTMENT, REGINA MARGHERITA CHILDREN'S HOSPITAL, CITY OF HEALTH AND SCIENCE OF TURIN, TURIN ITALY. PHONE: 3389351068 EMAIL: FABIO.TIMEUS@UNITO.IT **SPEAKER**
FEDERICA **TONOLO** DEPARTMENT OF COMPARATIVE BIOMEDICINE AND FOOD SCIENCE, UNIVERSITY OF PADOVA, VIALE DELL'UNIVERSITÀ 16, LEGNARO, 35020 (PD), ITALY PHONE: 3462196443 EMAIL: FEDERICA.TONOLO@UNIPD.IT **SPEAKER**	LUCAS **URBAN** DEPARTMENT OF PHARMACOLOGY, FACULTY OF MEDICINE, PAVOL JOZEF ŠAFÁRIK UNIVERSITY IN KOŠICE, KOŠICE, SLOVAK REPUBLIC PHONE: +421911863860 EMAIL:LURBAN@VUSCH.SK **POSTER PRESENTATION**

LUCA **VANNUCCI** INSTITUTE OF MICROBIOLOGY OF THE CAS, PRAGUE PHONE:+420731754022 EMAIL: VANNUCCI@BIOMED.CAS.CZ **SPEAKER**	TOMÁŠ **VASILENKO** DEPARTMENT OF SURGERY, AGEL HOSPITAL KOŠICE-ŠACA AND PAVOL JOZEF ŠAFÁRIK UNIVERSITY IN KOŠICE, KOŠICE, SLOVAK REPUBLIC PHONE +421908191144 EMAIL TOMASVASILENKO@GMAIL.COM **SPEAKER**
PROF PAVEL **VODICKA** INSTITUTE OF EXPERIMENTAL MEDICINE, ASCR PRAGUE, 2INSTITUTE OF BIOLOGY AND MEDICAL GENETICS, FIRST FACULTY OF MEDICINE, CHARLES UNIVERSITY, PRAGUE PHONE:00420724066944 EMAIL: PAVEL.VODICKA@IEM.CAS.CZ **SPEAKER**	HIRONOBU **WAKUNAGA** WAKUNAGA PHARMACEUTICAL CO. LTD HIROSHIMA, JAPAN EMAIL: WAKUNAGA_H@WAKUNAGA.CO.JP **ATTENDEE**
NING **WANG** AFFILIATED HANGZHOU FIRST PEOPLE'S HOSPITAL, ZHEJIANG UNIVERSITY SCHOOL OF MEDICINE, HANGZHOU, CHINA NO.261, HUANSHA ROAD, HANGZHOU, ZHEJIANG PROVINCE HANGZHOU/310000 CHINA PHONE: +8618668126835 EMAIL: NING8741@126.COM **SPEAKER**	MAKOTO **WATANABE** 2-1-1,TSUDAHIGASHIMACHI HIRAKATA, OSAKA 5730122 JAPAN PHONE: 0728589323 EMAIL: YODEN@HIRAKATARYOIKU-MED.OR.JP **ATTENDEE**
REIKO **WATANABE** 2-1-1,TSUDAHIGASHIMACHI HIRAKATA, OSAKA 5730122 JAPAN PHONE: 0728589323 EMAIL: HOISHI@HIRAKATARYOIKU-MED.OR.JP **ATTENDEE**	CHISATO **YAMANISHI** 2-1-1,TSUDAHIGASHIMACHI HIRAKATA, OSAKA 5730122 JAPAN PHONE: 0728589323 EMAIL: NAKANO@HIRAKATARYOIKU-MED.OR.JP **ATTENDEE**

HIROMICHI **YAMANISHI** 2-1-1,TSUDAHIGASHIMACHI HIRAKATA, OSAKA 5730122 JAPAN PHONE: 0728589323 EMAIL: YOSHIMIZU@HIRAKATARYOIKU-MED.OR.JP **ATTENDEE**	TANG **ZHU** AFFILIATED HANGZHOU FIRST PEOPLE'S HOSPITAL, ZHEJIANG UNIVERSITY SCHOOL OF MEDICINE, HANGZHOU, CHINA NO.261, HUANSHA ROAD, HANGZHOU, ZHEJIANG PROVINCE HANGZHOU/310000 CHINA **ATTENDEE**
WEI **ZHU** SCI RESEARCH INSTITUTE, 420 JERICHO TPKE, JERICHO, NY 11753 PHONE: 15164558438 EMAIL: ZHUWEI_69@YAHOO.COM **SPEAKER**	MATTEO **ZURLO** DEPARTMENT OF LIFE SCIENCES AND BIOTECHNOLOGY, UNIVERSITY OF FERRARA, FERRARA, ITALY EMAIL: MATTEO.ZURLO@UNIFE.IT **SPEAKER**

Abstracts

26th International Symposium on Molecular Medicine

2-4 November 2023

Grand Hotel Duca D'Este

Tivoli, Rome, Italy

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Melatonin and its metabolites: Major contributors to the maintenance of cellular redox homeostasis

Russel J Reiter
UT Health San Antonio, Long School of Medicine, San Antonio, TX, USA

Melatonin (N-acetyl-5-methoxytryptamine) was discovered as a secretory product of the mammalian pineal gland in 1958. At the time, melatonin was thought to be exclusively synthesized and released from the pineal gland at night with the duration of the nocturnal melatonin elevation being related to the length of the daily dark period. Since the duration of the night length varies seasonally, melatonin rhythm was originally believed to regulate only circadian (sleep/wake cycle) and circannual (annual reproductive fluctuations in seasonally breeding species) rhythms, i.e., to function as both a physiological clock and as a calendar. Subsequently, melatonin was also identified in non-mammalian vertebrates, all of which have a pineal gland, and soon thereafter in invertebrates including unicells and in plants, none of which have a pineal gland; thus, melatonin synthesis is obviously not exclusively of pineal origin. Within the last three decades, melatonin or its synthesizing enzymes have been found in prokaryotes including true bacteria and archaebacteria (archaeans). Thus, melatonin presumably evolved in prokaryotes about 3.0-2.5 billion years ago. During eukaryogenesis, which occurred an estimated 2.5 – 2.0 billion years ago, prokaryotes were engulfed by early eukaryotes for food and energy; the prokaryotes eventually established a symbiotic relationship with the eukaryotes that had phagocytized them, and they eventually evolved into mitochondria. The origin of mitochondria from prokaryotes is a widely accepted theory. The melatonin-synthesizing activity of the prokaryotes was retained in all eukaryotic mitochondria. As a result, the mitochondria of many cells of present-day eukaryotes may also produce melatonin; thus, vertebrates have two sources of melatonin. The pineal gland of vertebrates discharges melatonin into the blood and cerebrospinal fluid (the releasable pool) for circadian and circannual rhythm regulation. Additionally, all plant and animal species synthesize melatonin in their mitochondria, which is used as an antioxidant in the cell of origin (the non-releasable pool). Numerous publications have shown melatonin to be a multifaceted and highly efficient direct free radical scavenger and an indirect antioxidant. Melatonin quenches highly destructive reactive oxygen (ROS) and reactive nitrogen species as does many of its metabolites in what is referred to as the antioxidant cascade. Additionally, melatonin promotes the activities of numerous antioxidant enzymes while inhibiting pro-oxidant enzyme activities. Since mitochondria are a major source of ROS generation when electrons leak from complexes I and II of the electron transport chain and chemically reduce nearby ground-state oxygen to the superoxide anion radical, melatonin synthesis in mitochondria is ideally situated to reduce free radical damage. Supplemental melatonin limits elevated oxidative stress in many pathologies, such as in models of ischemia/reperfusion including stroke and heart attack, exposure of animals to heavy metals, environmental particulate matter, microplastics, hyperglycemia and atherosclerosis, excessive alcohol, conventional medications including chemotherapies, illegal drugs, ionizing radiation, neurodegenerative diseases, and those caused by dysfunctional mitochondria. These studies have been extended to humans where melatonin has similar protective actions. Some of the functions of melatonin involve transmembrane receptors which are widely distributed while other actions are receptor-independent.

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Inter and intra-tumoral heterogeneity of salivary adenoid cystic carcinoma: Implications for stratification and targeted therapy

Adel K. El-Naggar
M.D. Anderson Cancer Center, Houston, TX, USA

Adenoid cystic carcinoma is a common salivary gland cancer with propensity of recurrence and metastasis. The primary management of patients with this malignancy is a complete surgical resection with and without post-operative radiotherapy. Patients with recurrent and metastatic disease have limited therapeutic options. Although multiple clinical trials of conventional and targeted agents have been conducted, the results were unrewarding. Recently, extensive genomic studies have identified key fusion genes and genetic alterations that can be targeted by small molecule agents. The effectiveness of these agents in treatments of this disease depends on tumor genomic stability of these targets during ACC development and progression. The presentation will discuss recent data on the extent of intra- and inter-tumor heterogeneity of ACC and the implication of this inherent feature on patient's selection and stratification for therapy.

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Hemophagocytic lymphohistiocytosis in clinical practice: 18 years of experience of the Onco-Hematology Department of Regina Margherita Children's Hospital

Fabio Timeus[1,2], Veronica Barat[1], Nicoletta Bertorello[1], Elena Barisone[1], Elena Vassallo[1], Francesco Saglio[1], Maria Luisa Coniglio[3], Elena Sieni[3], Franca Fagioli[1]
[1]Pediatric Onco-Hematology Department, Regina Margherita Children's Hospital, City of Health and Science of Turin, Turin, Italy; [2]Pediatrics Department, Chivasso Hospital, Chivasso (TO), Italy; [3]Department of Pediatric Onco-Hematology, IRCCS Meyer Children's University Hospital, Florence, Italy
E-mail: fabio.timeus@unito.it

From September 2004 to November 2021, 39 patients aged 0- 17 years had a diagnosis of hemophagocytic lymphohistiocytosis (HLH) in our center. Fourteen patients were primary HLH (pHLH), 25 secondary HLH (sHLH). The mean age at diagnosis was respectively 1,74 years (range 0.09-13.2) for pHLH, 3.2 years (range 0.5-17.7) for sHLH. Time of follow-up was 5.63 years (range 0.53-17.42) for alive patients and 0.1 years for deceased patients (range 0.02-0.94). Median time for diagnosis from the onset of symptoms was 8 days (range 0-257). In the genetic forms, mutations involved *PRF1* in 4 patients, *UNC13D* in 3 patients, *SH2D1A* or *XIAP* in 4 patients, *RAB27A* genes in one patient and a ALPS mutation p.GLy66Cys associated with a monoallelic mutation on *PRF1* in one patient. The HLHs secondary to malignancies were the most frequent (56%): 7 cases associated with acute myeloid leukemia (AML), 4 cases with acute lymphoblastic leukemia (ALL) and 3 cases with non-Hodgkin lymphoma (NHL). In 4 patients HLH occurred after hematopoietic stem cell transplantation (HSCT), due to EBV reactivation in 3 patients and a Pneumocystis carinii infection in one patient. In one case, the diagnosis of HLH preceded the diagnosis of AML by weeks. Overall an infectious trigger was present in 28 out of 39 patients (72%): in 14 cases viral, in 4 cases bacterial, in 10 cases association of viruses and bacteria. The most numerous cases were due to EBV reactivation. Twenty-five patients (64%) were treated according to HLH protocols: HLH 94, HLH 2004 or corticosteroids associated with anti-thymocyte globulin (ATG). Specific chemotherapy for underlying malignancy was performed in 7 patients (18%). One patient was diagnosed with AML after 2 months of HLH therapy. For EBV reactivation we have used Rituximab in 5 cases. HSCT was performed in 18 cases (46%). Thirteen out of 14 FHL patients underwent HCST, one deceased for infectious complications before transplant procedure with FHL in complete remission (RC). In sHLH setting HSCT was performed in 5 patients: 1 for Niemann-Pick disease, 2 for anaplastic large lymphoma (ALCL) and 2 for high risk acute myeloid leukemia (AML). In addition to HLH diagnostic criteria 8 patients had SNC involvement (21%), 24 liver involvement (62%), 14 lung involvement (57,1%) and 2 cardiac involvement (5%). Overall survival (OS) and event free survival (EFS) at 5 years were respectively 64,1% ± 7,7 and 48,4%± 8,4. Favorable predictor factors were absence of SNC involvement, absence of heart involvement, ferritin level below median value of 6260 mcg/L and response at initial treatment. Our experience underlines the importance of always looking for an occult neoplasm even in infant HLH, when it is more probable a familial form. If HLH recurs, a review of the case and a new genetic testing is strongly recommended.

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The role of spermidine and its key metabolites in important, pathogenic human viruses and in parasitic infections caused by *Plasmodium*

Annette Kaiser
Medical Research Centre, University of Duisburg-Essen, Hufelandstrasse 55, 45147 Essen, Germany
E-mail: kaiser@microbiology-bonn.de

The triamine spermidine is a key metabolite of the polyamine pathway. It plays a crucial role in many infectious diseases caused by viral or parasitic infections. Spermidine and its metabolizing enzymes, i.e., spermidine/spermine-N^1-acetyltransferase, spermine oxidase, acetyl polyamine oxidase, and deoxyhypusine synthase, fulfill common functions during infection in parasitic protozoa and viruses which are obligate, intracellular parasites. The competition for this important polyamine between the infected host cell and the pathogen determines the severity of infection in disabling human parasites and pathogenic viruses. Here, we review the impact of spermidine and its metabolites in disease development of the most important, pathogenic human viruses such as SARS-CoV-2, HIV, Ebola, and in the human parasite *Plasmodium*. Moreover, state-of-the-art translational approaches to manipulate spermidine metabolism in the host and the pathogen are discussed to accelerate drug development against these threatful, infectious human diseases.

Keywords: spermidine; viral and parasitic infections; drug development

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Dose-dependent effect of polyamines on cancer cell proliferation and viability

T. Tahara[1,2], L. Di Magno[1], S. Coni[1], R. Bordone[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
[2]Department of Sensory Organs, Sapienza University of Rome
[3]International Polyamines Foundation 'ETS-ONLUS'
[4]Istituto Pasteur, Fondazione Cenci-Bolognetti, Sapienza University of Rome, Italy
E-mail: tomoaki.tahara@uniroma1.it

Spermidine (SPD) is a polyamine that is involved in various cellular processes, including proliferation, viability, and translation. Because cancer cells contain higher concentrations of polyamines than normal cells, their biosynthesis is considered an attractive target for cancer therapy. In contrast, toxic by-products of SPD, such as aldehydes including acrolein and H_2O_2, have been shown to induce cytotoxicity *in vitro,* and recent research has reported the potential use of spermidine to limit intestinal tumorigenesis *in vivo*[1]. Therefore, the administration of exogenous polyamines appears to induce distinct cellular outcomes in a concentration-dependent manner, although this issue has not been addressed in detail. Here, I investigated the mechanisms underlying changes in cell proliferation and viability associated with the administration of exogenous SPD in colorectal cancer cell lines. Upon polyamine depletion by DFMO, SPD treatment resulted in a dose-dependent, bidirectional effect. At lower doses (< 20 μM), SPD induced cell proliferation via DHPS and EIF5A hypusination. Conversely, at higher doses, SPD (> 100 μM) induced cytotoxicity independently of DHPS. Chloroquine, an autophagy inhibitor, ameliorated the cytotoxic effect of SPD, suggesting that SPD-induced cell death could be autophagy-dependent. Fetal bovine serum (FBS) in the culture medium was essential for the cytotoxic effect of high SPD as it contains bovine serum amine oxidase (BSAO), and the addition of exogenous BSAO, which has amine oxidase activity equivalent to that of FBS, restored SPD toxicity. Of note, the DHPS (Deoxyhypusine Synthase) inhibitor GC7 prevented the cytotoxic effect of high SPD by inhibiting BSAO in a non-competitive inhibitory manner, as evaluated by cell-free biochemical assays. Together, these results reveal the concentration-dependent survival and cytotoxic effects of SPD in cancer cells and provide a novel target for the widely used DHPS inhibitor, GC7.

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Expression of γ-globin genes in sirolimus-treated β-thalassemia patients

Cristina Zuccato[1,2], Lucia Carmela Cosenza[1], Matteo Zurlo[1], Alessia Finotti[1,2], Ilaria Lampronti[1,2], Monica Borgatti[1,2], Chiara Scapoli[1], Marco Prosdocimi[3], Maria Rita Gamberini[2] and Roberto Gambari[1,2]
[1]Department of Life Sciences and Biotechnology, Ferrara University, Ferrara; [2]Center Chiara Gemmo and Elio Zago for the Research on Thalassemia, Ferrara; [3]Rare Partners S.r.L., Impresa Sociale, Milano, Italy.
E-mail: gam@unife.it

The β-thalassemias are due to autosomal mutations in the gene encoding β-globin, causing the absence or low level of adult hemoglobin (HbA). High production of fetal hemoglobin (HbF) is beneficial for β-thalassemia patients. Sirolimus, also known as rapamycin, is a lipophilic macrolide isolated from a strain of *Streptomyces hygroscopicus* and found to be a strong inducer of HbF in vitro and in vivo. In this study, we report the major biochemical and molecular outcomes of the NCT03877809 pilot clinical trial based on the use of sirolimus. The results were obtained in 8 patients with β^+/β^+ and β^+/β^0 genotypes. The recruited patients were treated with a starting dosage of 1 mg/day sirolimus for 24-48 weeks. The first finding of the study was that the content of γ-globin mRNA, studied by RT-qPCR, was increased in blood and erythroid precursor cells isolated from sirolimus-treated β-thalassemia patients. A second important conclusion of the trial was that sirolimus stimulates HbF production (analyzed by HPLC) in erythroid cells of treated patients, influences erythropoiesis and reduces biochemical markers associated with ineffective erythropoiesis (such as the excess of free α-globin chains). The drug was well tolerated with minor effects on the immunophenotype, the only side effect being frequently occurring stomatitis. Despite the limited number of patients and the response variability, the data indicate that sirolimus given at low dosages modifies hematopoiesis and induces expression of γ-globin genes.

Supported by Wellcome Trust (United Kingdom, Innovator Award 208872/Z/17/Z), by the UE FP7 THALAMOSS Project (THALAssaemia MOdular Stratification System for personalized therapy of β-thalassemia; grant n.306201-FP7-Health-2012-INNOVATION-1) and by AIFA (Agenzia Italiana del Farmaco, Italy, AIFA-2016-02364887).

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Inhibitory effects of SARS-CoV-2 Spike protein and BNT162b2 vaccine on erythropoietin-induced globin gene expression in erythroid precursor cells (ErPCs) from β-thalassemia patients

Lucia Carmela Cosenza[1], Giovanni Marzaro[2], Matteo Zurlo[1], Jessica Gasparello[1], Cristina Zuccato[1], Alessia Finotti[1,3] and Roberto Gambari[1,3]
[1]Department of Life Sciences and Biotechnology, Ferrara University, Ferrara; [2]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova; [3]Center Chiara Gemmo e Elio Zago for the Research on Thalassemia, Ferrara, Italy
E-mail: gam@unife.it

During the recent COVID-19 pandemic several β-thalassemia patients have been infected by SARS-CoV-2 and most patients were vaccinated against the virus. Recent studies demonstrate an impact of SARS-CoV-2 infection on the hematopoietic system[1]. The main objective of this study was to verify the effects of exposure of erythroid precursor cells (ErPCs) from β-thalassemia patients to SARS-CoV-2 Spike protein (S-protein) and the BNT162b2 vaccine. Erythropoietin (EPO)-cultured ErPCs have been either untreated or cultured in the presence of S-protein or BNT162b2 vaccine. The employed ErPCs were from a β-thalassemia cellular Biobank developed before the COVID-19 pandemic[2]. The genotypes were β-IVSI-110/β-IVSI-110 (two patients) and $\beta^0$39/ $\beta^0$39 (one patient). The starting endogenous levels of HbF were 10.02-52.62% After 5 days, lysates were analyzed by HPLC, for analysis of hemoglobin production, and isolated RNA was assayed by RT-qPCR, for detection of the effects on globin gene expression.
The main conclusions of the results obtained are that SARS-Spike protein and BNT162b2-A vaccine inhibit (a) fetal hemoglobin production by β-thalassemic ErPCs and (b) inhibit γ-globin mRNA accumulation. In addition, we have performed in silico studies suggesting a high affinity of SARS-CoV-2 Spike protein to HbF. Remarkably, this affinity approaches the affinity of Spike to ACE2. Our results are consistent with the hypothesis of a relevant impact of SARS-CoV-2 infection and COVID-19 vaccination on the hematopoietic system.

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This work was funded by the MUR-FISR COVID-miRNAPNA Project (FISR2020IP_04128) (to A.F. and R.G.) and by the CARIPARO Foundation (MARZ_CARIVARI20_01 C94I20002500007, to G.M.).

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The Cystic Transmembrane Conductance Regulator Gene (CFTR) is under post-transcriptional control of microRNAs: effects of treatments with agomiRNAs and possible biomedical applications

Chiara Papi[1], Jessica Gasparello[1], Matteo Zurlo[1], Roberto Gambari[1,2] and Alessia Finotti[1,2]
[1]Department of Life Sciences and Biotechnology, Ferrara University; [2]Research Center on Innovative Therapies for Cystic Fibrosis, Ferrara University, Ferrara, Italy
E-mail: chiara.papi@unife.it

MicroRNAs are involved in the expression of the gene encoding the chloride channel CFTR (Cystic Fibrosis Transmembrane Conductance Regulator). The objective of this study is to compare the effects of pre-miR-145-5p, pre-miR-335-5p, and pre-miR-101-3p on CFTR in treated bronchial epithelial Calu-3 cells. CFTR mRNA was quantified by Reverse Transcription quantitative Polymerase-Chain Reaction (RT-qPCR); production of the CFTR protein was assessed by Western Blotting. The results obtained demonstrate that treatment with pre-miR-101-3p was inefficient, and treatment of Calu-3 cells with miR-145-5p was more effective in inhibiting CFTR production than treatment with the pre-miR-335-5p[1]. Treatment of target cells with the agomiRNA pre-miR-145-5p should be considered when CFTR gene expression should be inhibited in pathological conditions, such as Polycystic Kidney disease (PKD), some types of cancer, Cholera, and SARS-CoV-2 infection. For instance, CFTR inhibitors slow cyst growth in PKD[2], and the knockdown and/or inhibition of CFTR suppresses the proliferation of tumor cells *in vitro* and *in vivo*[3]. Finally, accordingly with the involvement of CFTR in the SARS-CoV-2 life cycle, pre-miR-145-5p was found to strongly inhibit viral entry and replication in infected Calu-3 cells[4].

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Inhibition of the expression of pro-inflammatory genes by pre-miR-93-5p: relevance for Cystic Fibrosis and COVID-19

Jessica Gasparello[1], Matteo Zurlo[1], Chiara Papi[1], Alessia Finotti[1,2], Enzo Agostinelli[3] and Roberto Gambari[1,2]
[1]Department of Life Sciences and Biotechnology, Ferrara University, Italy; [2]Research Center on Innovative Therapies for Cystic Fibrosis, University of Ferrara, Ferrara; [3]International Polyamines Foundation 'ETS-ONLUS', Rome, Italy
E-mail: alessia.finotti@unife.it

We have analyzed the effects of transfecting cystic fibrosis (CF) bronchial epithelial IB3-1 cells with pre-miR-93-5p, mimicking the biological activity of miR-93-5p, found downregulated in IB3-1 cells infected with *Pseudomonas aeruginosa*[1], in parallel with increased expression of miR-93 target mRNAs, the most relevant being IL-8 mRNA. The results obtained support the concept of a possible link between the expression of miR-93-3p and IL-8 induction in bronchial epithelial cells infected with *P. aeruginosa*. Specifically, the data obtained indicate that, in addition to NF-κB-dependent up-regulation of IL-8 gene transcription, IL-8 protein expression is post-transcriptionally regulated by interactions of the IL-8 mRNA with the inhibitory miR-93-5p. In a second set of experiments, we have exposed IB3-1 cells to the SARS-CoV-2 Spike protein in the absence or in the presence of transfected pre-miR-93-5p. The results obtained demonstrate that the production of IL-6, IL-8 and G-CSF proteins is enhanced in IB3-1 cells by treatment with the SARS-CoV-2 Spike protein and that their synthesis and extracellular release can be strongly reduced using the agomiRNA molecule mimicking miR-93-5p.
In conclusion, the release of key proteins of the COVID-19 "cytokine storm" can be inhibited by mimicking the biological activity of microRNAs.

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This work was funded by the MUR-FISR COVID-miRNAPNA Project (FISR2020IP_04128) (to A.F. and R.G.)

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Antiviral activity of AGE and SAC against different viruses: preliminary study

Chiara Nonne[1], Roberta Campagna[1], Daniele Emanuele Compagnino[1], Maria Gemma Leone[1], Germana Sfara[1], Gianluca Canettieri[2], Ombretta Turriziani[1], Enzo Agostinelli[3]
[1]Department of Molecular Medicine, Sapienza University of Rome, Viale dell'Università 31, 00185, Rome, Italy; [2]Department of Molecular Medicine, Sapienza University of Rome, Laboratory Affiliated to Istituto Pasteur Italia, Fondazione Cenci-Bolognetti, Viale Regina Elena 291, 00161, Rome, Italy; [4]International Polyamines Foundation-ONLUS, Via del Forte Tiburtino 98, 00159, Rome, Italy and Department of Sensory Organs, Sapienza University of Rome, Policlinico Umberto I, Viale del Policlinico 151, 00155, Rome, Italy
E-mail: chiara.nonne@uniroma1.it

Garlic and garlic extracts, especially aged garlic extract (AGE), are rich in bioactive compounds, with potent anti-inflammatory, antioxidant and neuroprotective activities (1). Notably, AGE has been demonstrated to exert an anti-proliferative effect on different human cancer cells (2). In light of these findings, we investigated the potential antiviral activity of AGE and one of its derivative S-allyl-cysteine (SAC) on different viruses. Cytotoxic activity of substances was evaluated on VERO-E6 and A549 cells by an MTT assay. Antiviral activity was tested against Coxsackievirus B6, Vescicular stomatitis virus (VSV) and Encephalomycarditis virus (EMCV). The percentage of viral replication inhibition was calculated by measuring the virus yield in the supernatant of infected cells. No cytotoxic effect was found at concentration used for AGE (5 mg/ml, 1 mg/ml, 0.2 mg/ml, 0.04 mg/ml); and SAC (100 µM, SAC 20 µM, SAC 4 µM, SAC 0.8 µM). In VERO-E6 cells AGE 1mg/ml was able to inhibit Coxsackievirus B6, and VSV by 76% and 84% respectively. No viral replication inhibition was observed with EMCV. While SAC 100 µM was able to inhibit Coxsackievirus B6, VSV and EMCV by 85%, 95% and 87% respectively on the same cell line. On the contrary in A549 cells AGE 1mg/ml and SAC 100 µM were able to inhibit only EMCV by 97%. Interestingly, viral replication reduction was observed only when absorption and penetration of viruses occurred in the presence of AGE or SAC, suggesting that these compounds could exert their antiviral activity in the early phases of viral replication.

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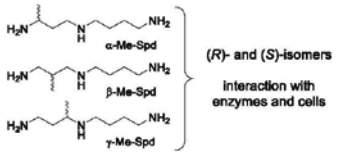
C-Methylated analogues of spermidine: synthesis and biological application

Arthur I. Salikhov, Maxim A. Khomutov
Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia
E-mail: makhomutov@mail.ru

The biogenic polyamines spermine (Spm) and spermidine (Spd) are essential and ubiquitous organic polycations present in all eukaryotic cells in micromolar to millimolar concentrations, which *a priori* determines the diversity of their functions, many of which are vitally important[1,2]. The disturbances of polyamines metabolism are associated with many diseases, including cancer (tumor cells have elevated concentration of polyamines). The investigation of individual cellular functions of Spm and Spd is complicated by their interchangeability and the ease of their interconversion.
Different polyamine structural analogues have been designed for the research purposes and as potential therapeutic compounds. Terminally *bis*-*N*-alkylated analogues, such as N^1,N^{11}-diethylnorspermine (DENSpm), are among the most studied potential polyamine-based cancer chemotherapeutics. *C*-Methylated polyamine analogues are unique, because among these compounds functionally active and metabolically stable *in vitro* and *in vivo* mimetics of Spd and Spm were found. Biochemical properties of these compounds can be regulated by moving methyl substituent along the polyamine backbone. Furthermore, enzymes of polyamine metabolism have been shown to possess hidden stereospecificity which makes it possible to modulate the biological activity of *C*-methylated polyamine analogues by changing the stereo-configuration of chiral centers[3].



Different synthetic approaches for the preparation of the isomers of *C*-methylated Spd's, their interaction with the enzymes of polyamine metabolism and antizyme-related effects are discussed[4].

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New weapons against multidrug resistant bacterial infections via an RNA therapeutics antibiotic drug discovery platform

Peter E. Nielsen
Center for Peptide-Based Antibiotics, Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Multidrug-resistant Gram-negative bacteria pose an increasing threat to human health, and development of novel antibiotics would be one answer to this challenge. Most efforts to date have focused on development of broad-spectrum antibiotics and unfortunately with limited success in terms of approved new drugs. As an alternative approach we have developed an RNA therapeutics platform based on PNA antisense technology that allows rational discovery and development of designer precision, narrow spectrum antibiotics optimized to target different multidrug resistant bacterial species using the same platform and principle. Using this platform, PNA antisense antibiotics (PAAs) showing (sub)micromolar antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* (including multidrug resistant clinical isolates) have been discovered. Lead compounds are bactericidal via and antisense mechanism of action, exhibit low frequency of resistance, have exquisite biostability in human (and mouse) serum, very low toxicity in human cell culture (HepG2), good *in vivo* tolerability in mice via *sc*, *iv* and *ip* administration. Finally, *in vivo* efficacy against MDR *E. coli* and *A. baumannii* in infection mouse models of urinary tract infection, sepsis and soft tissue infection has been demonstrated. Based on these *in vitro* as well as *in vivo* results the prospects of developing novel precision antibiotics against infections by multidrug resistant Gram-negative bacteria will be discussed.

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The sweet side of wound healing: galectins as promising therapeutic targets in the treatment of open excisions and sutured incisions

Vlasta Gálová[1,2], Tomáš Vasilenko[3], Lukáš Urban[1,4], Karel Smetana Jr.[5], Herbert Kaltner[6], Peter Gál[1,4]
[1]Department of Biomedical Research, East-Slovak Institute of Cardiovascular Diseases, Inc., Košice, Slovak Republic
[2]Department of Pharmacy, Children University Hospital, Košice, Slovak Republic
[3]Department of Surgery, Agel Hospital Košice-Šaca and Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic
[4]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic
[5]Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic
[6]Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilian-University, Munich, Germany
E-mail: vlasta.perzelova@gmail.com

Understanding the molecular processes involved in wound healing may pave the way for the development of innovative approaches to transforming the identified natural effectors into therapeutic tools. Based on the extensive involvement of the ga(lactoside-binding)lectin family in (patho) physiological processes, it has been well established that galectins are involved in a wide range of cell-cell and cell-matrix interactions (1). Therefore we treat cells involved in wound repair as well as Sprague-Dawley rats to reveal the therapeutic potential of two members of the galectin family, i.e. Gal-1 and Gal-3. The reported data make a strong case for directing further efforts to treat excisional and incisional wounds differently. Functions of galectins essentially result from their modular presentation. In fact, Gal-1 seems to play a role in the early phases of healing and wound contraction, Gal-3 accelerates re-epithelization and increases tensile strength (2). Galectins have also become subject of redesigning by engineering to optimize the activity. Clinically relevant, these new tools derived from the carbohydrate recognition domain platform also proved efficient on the in vitro level (SMAD-signaling) and will become our target in further animal studies.

Acknowledgement: The study was supported in part by the Grant Agency of the Ministry of the Education, Science, Research and Sport of the Slovak Republic (VEGA 1/0319/20 and 1/0455/22), and the Slovak Research and Development Agency (APVV-20-0017).

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Novel approaches to treat wounds

Peter Gál[1,2,3], Tomáš Vasilenko[4], David Lukáš[5], Robert Zajíček[3], Dominik Rejman[6]
[1]Department of Biomedical Research, East-Slovak Institute of Cardiovascular Diseases, Inc., Košice, Slovak Republic
[2]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic
[3]Prague Burn Center, Third Faculty of Medicine, Charles University and University Hospital, Prague, Czech Republic
[4]Department of Surgery, Agel Hospital Košice-Šaca and Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic
[5]Faculty of Science, Humanities and Education, Technical University of Liberec, Liberec, Czech Republic
[6]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic
E-mail: galovci@yahoo.com

Poor skin wound healing significantly increases health-care costs. Active wound dressings are attracting extensive attention in wound treatment including bacteria-infected lesions. As the wide use of antibiotics leads to drug resistance we present here a new concept of wound dressing based on the polycaprolactone nanofiber scaffold (NANO) releasing second generation lipophosphonoxin (LPPO) as antibacterial agent 1. Firstly, we demonstrated in vitro that LPPO released from NANO exerted antibacterial activity while not impairing proliferation/differentiation of fibroblasts and keratinocytes. Secondly, using animal model we showed that NANO loaded with LPPO significantly reduced the Staphylococcus aureus counts in infected wounds. Furthermore, the rate of degradation and subsequent LPPO release in infected wounds was also facilitated by lytic enzymes secreted by inoculated bacteria. LPPO displayed negligible to no systemic absorption. In conclusion, the composite antibacterial NANO-LPPO-based dressing reduces the bacterial load and promotes skin wound healing, thus may present a novel product improving patient`s care in the near future.

Acknowledgement: The study was supported in part by the Grant Agency of the Ministry of the Education, Science, Research and Sport of the Slovak Republic (VEGA 1/0319/20 and 1/0455/22), and the Slovak Research and Development Agency (APVV-20-0017).

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Agrimonia eupatoria L. and skin wound healing

Tomáš Vasilenko[1], Ivan Kováč[2], Andrej Vrzgula[1], Karel Smetana Jr[3], Peter Gál[4,5]
[1]Department of Surgery, Agel Hospital Košice-Šaca and Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic
[2]Second Department of Surgery, Louis Pasteur University Hospital and Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic
[3]Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic
[4]Department of Biomedical Research, East-Slovak Institute of Cardiovascular Diseases, Inc., Košice, Slovak Republic
[5]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic
E-mail: tomasvasilenko@gmail.com

Previously, we demonstrated that Agrimonia eupatoria L. (AE) is a valuable source of polyphenols with excellent antioxidant properties (1). Inspired by our previously published data (2), in the present study we further examined whether AE improves skin wound healing in a series of *in vitro* and *in vivo* experiments. In detail, we investigated the cytotoxicity of AE extracts on fibroblasts, keratinocytes and endothelial cells. We also demonstrated the ability of the extract to induce fibroblast to myofibroblast conversion, extracellular matrix (ECM) deposition, and keratinocyte proliferation. The cytotoxicity assay revealed comparable concentration-dependent effects between studied extracts, thus we used the water extract for the animal study. Subsequently, in Sprague-Dawley rats, we measured wound tensile strength (TS) and assessed the progression of open wounds using basic histology and immunofluorescence. The AE extract induced the myofibroblast-like phenotype and enhanced ECM deposition. Furthermore, the wound TS of skin incisions and the contraction rates of open excisions were significantly increased in the AE-treated group. The present data revealed that AE water extract significantly improves wound healing. From this point of view, our data warrant further testing in animal models closer to humans.

Acknowledgement: The study was supported in part by the Grant Agency of the Ministry of the Education, Science, Research and Sport of the Slovak Republic (VEGA 1/0319/20 and 1/0455/22), and the Slovak Research and Development Agency (APVV-20-0017).

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LncRNA MALAT1, post-transcriptionally stabilized by NSUN2-mediated M5C modification, exerts properties in bone lesions formation in multiple myeloma

Xing Cui[1#*], Manya Yu[2#]
[1]Department of Oncology and Hematology, the Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250001, P.R. China; [2]College of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, 16369 Jingshi Road, Jinan 250014, P.R. China
*Corresponding author email: cdz45@foxmail.com
#These authors made equal contributions to this work

Multiple myeloma (MM) is still an incurable disease and osteoclast-mediated bone destruction is a hallmark. Our study found that lnc MALAT1 expression was the highest in exosomes derived from MM cells, and it was significantly increased in MM cells compared with normal plasma cells. Then, we labeled isolated exosomes with PKH26 dye and MALAT1 with GFP from U266 cells. It suggested that MALAT1 could be packaged into exosomes and swallowed by RAW264.7 cells. Overexpression of MALAT1 could upregulate RANKL expression and increase TRAP-positive osteoclasts (OCs) and mineralized nodules, while the knockdown of MALAT1 showed a reverse trend. The ability of MM exosomes in bone destruction was then verified in vivo by injecting U266 cells, U266 cells plus MM exosomes, and U266 cells plus MALAT1-knockdown-exosomes into the tail vein of NOG mice. Parametric analysis using microCT revealed a significant decrease in the bone volume by exosomes mediated MALAT1. Recent studies focused on the function of RNA modification in regulating lncRNAs expression. Knockdown of NSUN2 significantly reduced the m5C levels of MALAT1. A rescue experiment showed that MALAT1 expression and stability increased by NSUN2 were reversed when transfected with YBX1 siRNA. Our study demonstrates that myeloma cells-derived exosomal lncRNA MALAT1 might involve in bone lesions formation, which is modulated by NUNS2-mediated m5 C modifications.

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The beneficial effects of novel telomerase increasing compounds on neurodegenerative and ageing related diseases

Esther Priel
Faculty of Health Sciences, the Ben-Gurion University of the Negev, and Neuromagen Pharma Ltd., Beer-Sheva, Israel

Telomerase, a ribonucleoprotein, mainly consists of the telomerase reverse transcriptase catalytic subunit (TERT) and the RNA template (TERC). The canonical function of telomerase is to re-elongate telomeres, at the end of the chromosomes, and thus increase the life span of cells and tissue. In addition TERT possesses non-canonical functions such as: gene transcription regulation, participates in DNA repair and signaling pathways and protects the mitochondria from oxidative stress. TERT is expressed in proliferating tissue and in human somatic cells it is undetectable. Its functions in the extension of life span and in the repair and protection of cells from damages, are the basis for the notion that a transient and controlled increase of TERT expression and activity will demonstrate significant beneficial effects on cells and tissue, under normal and specifically under stress conditions. We synthesized novel small molecules (AGS) that transiently increased TERT expression and telomerase activity in vitro and in vivo in various mouse and human cells and tissues. We found that some of the AGS compounds crossed the blood-brain -barrier and increased TERT expression in the mouse brain. Daily administration of the AGS compounds to neurodegenerative mouse models (ALS, Alzheimer) delayed the onset and progression of the ALS symptoms in ALS mice, and significantly decreased the burden of the amyloid beta plaques in the brain of AD mice. The beneficial effects of increasing TERT are also shown in the pancreas of diabetic rats and mice. We found that AGS compounds administered to animals with STZ-induced diabetes, after the establishing of hyperglycemia, significantly increased/restored the number of the insulin producing beta cells, decreased the blood glucose level after fast and protected the kidney from some of the diabetes -induced damages. Previous studies suggested the possible role of telomerase in female and male fertility. We found that a transient increase of TERT by a single dose of AGS significantly improved fertility in female and in male mice. The observed biological effects of AGS compounds are TERT dependent since no effects were detected in TERT knock-out mice. Long-term treatment with these compounds did not cause chromosomal aberrations in human cells. Our data strongly suggest the importance of a controlled increase of TERT by pharmaceutical compounds like AGS for the treatment of neurodegenerative and ageing related diseases.

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Phosphorus analogues of glutamic acid and *S*-adenosylmethionine: Synthesis and biological activity

Alex R. Khomutov[1], Fabio Giovannercole[2], Marija V. Demiankova[3], Vsevolod L. Filonov[1], Maxim A. Khomutov[1], Olga V. Efremenkova[3], Daniela De Biase[2]
[1]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia; [2]Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy and [3]Gause Institute of New Antibiotics, Moscow, Russia
E-mail: alexkhom@list.ru

The replacement of the carboxyl group of amino acids with an acidic phosphorus containing group(s) gives rise to a big family of the compounds, but only single-charged phosphorus containing group of aminophosphinic acids (X=H) is a flatted tetrahedron and a mimetic of a planar single-charged carboxyl group. Therefore, some aminophosphinic acids can undergo substrate-like transformations and may inhibit cell growth not only as such, but also due to the formation of new biologically active metabolites. Phosphorus containing analogues of *S*-adenosylmethionine, glutamate and dipeptides of the last one were synthesized:
The interaction of these analogues with some enzymes of glutamate and *S*-adenosylmethionine metabolism is discussed, as well as their antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and multidrug-resistant *Klebsiella pneumoniae*.

Supported by the Russian Science Foundation grant no. 22-14-00291.

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Autophagy promotes insulin secretion under glucose stimulation both *in vitro* and *in vivo* in a RAB37 dependent manner

Shan-Ying Wu[1], Yi-Ching Wang[2], Sheng-Hui Lan[3], Hsiao-Sheng Liu[4,5]
[1]Department of Microbiology and Immunology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan; [2]Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan, Taiwan; [3]Department of Life Sciences and Institute of Genome Sciences, National Yang Ming Chiao Tung University; [4]Center for Cancer Research, Graduate Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University; [5]M.Sc. Program in Tropical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.
E-mail: hsliu713@kmu.edu.tw

Insulin is the key regulatory hormone for glucose homeostasis. Aberration of insulin glycose was diagnosed in pancreatic β-cells exhibiting autophagy deficiency; however, the underlying mechanism remains unknown. We reveal that increased macroautophagic/autophagic activity leads to increase of insulin secretion in β-cells both *in vivo* and *in vitro* under high-glucose conditions. Moreover, proteomic analysis of purified autophagosomes from β-cells identified a group of vesicular transport proteins participating in insulin secretion, implying that secretory autophagy regulates insulin exocytosis. RAB37, a small GTPase, regulates vesicle biogenesis, trafficking, and cargo release. We demonstrated that the active form of RAB37 increased MAP1LC3/LC3 lipidation (LC3-II) and is essential for the promotion of insulin secretion by autophagy, but these phenomena were not observed in *rab37* knockout *(rab37-/-)* cells and mice. Unbalanced insulin and glucose concentration in the blood was improved by manipulating autophagic activity using a novel autophagy inducer niclosamide (an antihelminthic drug) in a high-fat diet (HFD)-obesity mouse model. In summary, we reveal that secretory autophagy promotes RAB37-mediated insulin secretion to maintain the homeostasis of insulin and glucose both *in vitro* and *in vivo*.

(1) Wu et al., Autophagy, https://doi.org/10.1080/15548627.2022.2123098

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Aqueous ozone exhibits anticancer activity by triggering mitochondria-targeted oxidative cell death

Manami Suzuki-Karasaki[1,2], Yushi Ochiai[1], Shizuka Innami[1], Hiroshi Okajima[3], Miki Suzuki-Karasaki[1], Hideki Nakayama[4], and Yoshihiro Suzuki-Karasaki[1]
[1]Plasma ChemiBio Laboratory, Nasushiobara, Tochigi; [2]Graduate School of Medical Sciences, Kumamoto University, Kumamoto; [3]Communication &Control Systems Company, Tokyo Keiki Incorporation, Tokyo; [4]Department of Oral and Maxillofacial Surgery, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan
E-mail: ksmanami181@gmail.com

O_3 gas has shown the anticancer effects of O_3. It has a direct antitumor effect in some cancers and indirect effects, such as immunomodulatory, synergistic, or adjuvant effects, with various anticancer drugs and radiation. In contrast, the anticancer capacity of O_3 in solutions is obscure because most ozonated solutions also contain potentially cytotoxic nitrogen oxides (NOx). To address this question, we produced an aqueous O_3 free from NOx (ODM). ODM exhibited anticancer activity against various cancers by triggering cell death. Mitochondrial oxidative stress (mitoOS), mitochondrial dynamics and positioning alterations, and nuclear damage proceeded. Cell death and nuclear injury were prevented by iron chelators and catalase and accompanied by the remodeling of intracellular labile iron pools, including mitochondrial iron. ODM also increases nuclear and nucleolar ROS and their morphological changes. Scavenging ROS entirely attenuated mitochondrial and nuclear oxidative stresses and morphological alterations. These results suggest that O_3 in solutions exhibits anticancer activity by increasing mitoOS. Our findings imply that mitoOS is a potential cause of altered redox equilibrium in the nucleus and nucleolus, leading to multi-organellar damage and cell death.

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Canonical and non-canonical transforming factor-beta signaling in fibroblasts isolated from various neoplastic/healing/normal tissues

Matúš Čoma[1,2]
[1]Department of Pharmacology, Faculty of Medicine, University of Pavol Jozef Safarik in Kosice, Kosice; [2]Deparment for Biomedical Research, East-Slovak Institute of Cardiovascular Diseases, Inc., Kosice
E-mail: coma.matus@gmail.com

Transforming Growth Factor-beta (TGF-β) is a key molecule in wound healing as it drives the deposition of extracellular matrix (ECM), fibrosis, and epithelial-to-mesenchymal transition (EMT). While previously considered a simple non-amplified signaling pathway, recent research has shown that TGF-β signaling is more complex, involving both canonical (SMAD2/3) and non-canonical (Erk1/2, JNK, p38, Akt, ROCK) pathways that play important roles in fibrosis.The similarities between wound healing and tumor progression have led us to study the cellular response of fibroblasts isolated from various neoplastic, healing, and normal tissues to TGF-β. Specifically, we focused on signaling pathways activation by TGF-β1 and -β3, as well as the resulting changes in induced phenotype shifts. Understanding the complex signaling pathways involved in TGF-β signaling and the resulting changes in cellular phenotype can have important implications for both wound healing and cancer treatment. In the context of wound healing, targeting specific TGF-β signaling pathways could potentially enhance the healing process and reduce the formation of scar tissue. In the context of cancer treatment, targeting TGF-β signaling pathways could potentially inhibit tumor growth and metastasis.

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Effects of high intensity interval vs. low intensity continuous training on LXRβ, ABCG5 and ABCG8 genes expression in male Wister rats

Sediqeh Jalali[1], Mohsen Jafari[2]
[1]Department of Biology, Payam Noor University, Po Box 4697-19395, Tehran, Iran
[2]Department of Sport Sciences, Shirvan Branch, Islamic Azad University, Shirvan, Iran
E-mail: sediqeh.jalali4@gmail.com

Liver X receptors (LXR) have an essential role in the regulation of cholesterol metabolism and their activation increase ABCG5 and ABCG8 genes expression for the improvement of cholesterol excretion from body during reverse cholesterol transport (RCT). The aim of this study was to investigate the effects of high intensity interval (HIT) and low intensity continuous (LIT) trainings on gene expression of these substances after a high fat diet in Wistar rats.
Materials and Methods: Fifteen male Wistar rats were divided into 3 groups: control group (n = 5), HIT exercise group (n = 5) and LIT exercise group (n = 5). All groups used a high fat diet for 13 weeks and the HIT and LIT groups performed the specific training program. The expression of LXRβ, ABCG5 and ABCG8 genes was measured after the training period.
Findings: Data analysis showed significant higher levels of LXRβ, ABCG5 and ABCG8 genes expression in HIT and LIT groups compared to the control group ($P \leq 0.05$).
Conclusion: HIT and LIT trainings after a high fat diet have beneficial effects on RCT that prevents heart attack. Also, HIT training may have a greater effect on cholesterol excretion during the reverse cholesterol transport mechanism than LIT.

Keywords: Liver X Receptor, Atherosclerosis, Interval Training, Endurance Training

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Mitochondrial dysfunction in Down syndrome is caused by an alteration in the NRIP1/PGC-1α pathway and occurs early during neuronal differentiation

Nunzia Mollo, Adriana Limone, Matteo Esposito, Ferdinando Bonfiglio, Claudio Procaccini, Agnese Secondo, Gaetano Calì, Daniela Sarnataro, Simona Paladino, Anna Conti, Gabriella De Vita, Lucio Nitsch, Antonella Izzo
Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, 80131 Naples, Italy

Mitochondrial dysfunction has been observed in cells and tissues in Down syndrome (DS). In the effort to understand its causes we identified the corepressor NRIP1, a chromosome 21 gene that is upregulated in trisomic cells as a consequence of gene dosage and has among its targets PGC-1α, a master gene in mitochondrial biogenesis and function. Knockdown of NRIP1 can restore the level and function of PGC-1α protein and counteracts mitochondrial dysfunction in trisomic fibroblasts. A similar result can be achieved using the drug metformin, which activates PGC-1α. To understand when mitochondrial alterations occur, we differentiated iPSCs with trisomy of chromosome 21 into cortical neurons and monitored the mitochondrial phenotype. We found that mitochondrial dysfunction manifests at early stages of neuronal differentiation of trisomic iPSCs. Our data indicate that mitochondrial dysfunction in DS can be counteracted and suggest that it is among the possible causes of altered neuronal differentiation.

Down syndrome, NRIP1, PGC-1α, metformin, mitochondrial phenotype, neuronal differentiation

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Gene editing for the therapy of β-thalassemia: combining CRISPR-Cas9-based gene correction and fetal hemoglobin induction

Lucia Carmela Cosenza, Cristina Zuccato C, Matteo Zurlo, Roberto Gambari and Alessia Finotti
Center Chiara Gemmo and Elio Zago for the Resaerch on Thalassemia, University of Ferrara, Ferrara, Italy
E-mail: alessia.finotti@unife.it

Gene editing (GE) is an efficient strategy for correcting genetic mutations in monogenic hereditary diseases, including β-thalassemia. Recently, several different therapeutic protocols already used in clinical trials proposed the reactivation of fetal hemoglobin (HbF) production in β-thalassemia hematopoietic stem cells, obtained using the CRISPR-Cas9-based gene editing approach. We have reported that CRISPR-Cas9-based gene editing can also be employed for the efficient correction of the β039-thalassemia mutation, by directly modifying the mutated sequence on the β-globin gene1. On the other hand, considering that the increased production of HbF can be beneficial for patients with β-thalassemia, the aim of our study was also to verify whether the *de novo* production of adult hemoglobin (HbA) using CRISPR-Cas9 gene editing can be combined with HbF induction protocols using known and novel fetal hemoglobin inducers drugs2. The gene editing of the β039-globin mutation was obtained using a CRISPR-Cas9-based experimental strategy; the correction of the gene sequence and the transcription of the corrected gene were analyzed by allele-specific droplet digital PCR and RT-qPCR, respectively; the relative content of HbA and HbF was studied by high-performance liquid chromatography (HPLC) and Western blotting. For HbF induction, the repurposed drug rapamycin was used. The data obtained conclusively demonstrate that the maximal production of HbA and HbF is obtained in GE-corrected, rapamycin-induced erythroid progenitors isolated from β039-thalassemia patients. In conclusion, GE and HbF induction might be used in combination to achieve the *de novo* production of HbA together with an increase of induced HbF.

(1) Cosenza et al. Mol Ther Methods Clin Dev. 2021;21:507-523; (2) Cosenza et al. Genes (Basel). 2022;13(10):1727
This work was supported by Wellcome Trust Innovator Award (208872/Z/17/Z), by the UE FP7 THALAMOSS Project (n.306201-FP7-Health-2012-INNOVATION-1), and by AIFA (Agenzia Italiana del Farmaco, AIFA-2016-02364887).

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Novel stevioside derivative induced apoptosis in colon cancers cells via mitochondrial signaling pathways

Ahmed Malki[1] and Ahmed El Sharkawy[2]
[1]Department of Biomedical Sciences, College of Health Sciences, QU Health, Qatar University, Doha, Qatar; [2]Human Molecular Genetics Laboratory, Institute of Genetics and Biophysics "A. Buzzati-Traverso" (IGB)-CNR, Naples, Italy
E-mail: ahmed.malki@qu.edu.qa

Colorectal cancer (CRC) is the second most common cancer worldwide. In this study, we screened 20 steviosides derivatives for their cytotoxic activities in RKO colon cancer cells. We have identified a potent stevioside derivative 8 which induced apoptosis in RKO cells and results are further confirmed by TUNEL assay. We investigated some of the molecular activity of the key apoptotic signaling pathways, our data revealed that stevioside derivative 8 increased expression level of p53, cyclin D1, Bad and caspase-8 while it reduced the expression level of STAT-3, Akt, p MAPK and MDM2. To investigate the mitochondrial apoptotic events, stevioside derivative 8 increased protein levels of Bax, decreased Bcl-2 level and increased release from cytochrome c from mitochondria to cytoplasm which is further confirmed by immuohistochemical analysis. Furthermore, stevioside derivative 8 significantly increased caspase 9 levels and when RKO cells pretreated with caspase-9 inhibitor LEHD, the induction of apoptosis was significantly reduced. Finally, stevioside derivative 8 increased caspase-3 activities and Parp-1 cleavage. Our results suggest that stevioside derivative 8 is considered as promising anticancer agent targeting mitochondrial pathways which merits further development and investigations.

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Polyamine/EIF5A axis in colorectal tumors

Sonia Coni[1], Rosa Bordone[1], Devon Michael Ivy[1], Zuleyha Nihan Yurtsever[1], Laura Di Magno[1], Rodrigo D'Amico[1], Bianca Cesaro[3], Alessandro Fatica[3], Francesca Belardinilli[1], Francesca Bufalieri[1], Marella Maroder[1], Enrico De Smaele[4], Lucia Di Marcotullio[1], Giuseppe Giannini[1], Enzo Agostinelli[5], Gianluca Canettieri[1]
[1]Department of Molecular Medicine, Sapienza University of Rome, Laboratory Affiliated to Istituto Pasteur Italia, Fondazione Cenci-Bolognetti, Viale Regina Elena 291, 00161, Rome, Italy
[2]Department of Biochemical Sciences "A. Rossi Fanelli", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy
[3]Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy
[4]Department of Experimental Medicine, Sapienza University, 00161, Rome, Italy
[5]International Polyamines Foundation-ONLUS, Via del Forte Tiburtino 98, 00159, Rome, Italy; Department of Sense Organs, Sapienza University of Rome, Policlinico Umberto I, Viale del Policlinico 151, 00155, Rome, Italy
Email: Rosa.bordone@uniroma1.it the irreversible ODC inhibitor DFMO

Colorectal cancer (CRC) is the third-most lethal cancer worldwide. Pathways commonly altered in CRC converge on the activation of the MYC oncogene, suggesting the importance of its targeting. However, attempts to directly inhibit MYC have been unsuccessful, hence preferable options could be represented by inhibition of processes regulated by its function, such as polyamine metabolism. Polyamines are small polycations often increased in cancer and involved in many cellular functions included the post-translational modification of EIF5A, a translation factor that alleviates ribosome stalling at specific pausing motifs. Spermidine, in fact, is converted into hypusine and covalently bound to EIF5A by two enzymes (DHPS and DOHH). Recent studies have shown that EIF5A is overexpressed in various cancers, including CRC, where it correlates with poor prognosis. Also, previous reports have documented the therapeutic efficacy achieved by inhibition of hypusination with the specific DHPS inhibitor GC7 in some cancers. However, the actual mechanism of action and the direct translational targets of DHPS-EIF5A axis, responsible for the tumor-promoting effects, remain largely unknown. A key role in controlling the rate of polyamine production is played by ornithine decarboxylase (ODC). The irreversible ODC inhibitor DFMO was shown to efficiently prevent tumor growth in different settings, leading to clinical trials in colorectal cancer patients. However, the therapeutic benefit of this approach was limited, mainly because of the occurrence of resistance due to the reconstitution of intracellular polyamine content from the extracellular pool caused by compensatory upregulation of polyamine transporters and increased polyamine uptake. We have studied the effect of DHPS-EIF5A axis inhibition on CRC growth alone or in combination with DFMO to try to overcome the chemoresistance to prolonged ODC inhibition to treat CRC. Our work has led to the identification of MYC as a key translational target of hypusinated EIF5A and to the demonstration that inhibition of DHPS efficiently suppresses CRC cell growth and intestinal tumorigenesis in mice, by directly inhibiting eIF5A-mediated elongation of MYC at five distinct pausing motifs in MYC coding sequence (CDS). Moreover, we have demonstrated that combined inhibition of ODC and eIF5A induces a synergistic antitumor response in CRC cells, leading to complete suppression of MYC translation in a bimodal fashion, by preventing translational elongation and initiation. We found that genes of the polyamine biosynthesis and hypusination pathways are significantly upregulated in colorectal cancer patients and that inhibition of ODC or DHPS alone limits CRC cell proliferation through a cytostatic mechanism, while combined ODC and DHPS/EIF5A blockade induces a synergistic inhibition, accompanied to apoptotic cell death in vitro and in mouse models of CRC and FAP. Together, our data illustrate a novel strategy for CRC treatment, based on the combined suppression of ODC and eIF5A which hold promise for the treatment of CRC.

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Research and analysis on CT signs and clinical characteristics of chronic duodenal papilla mucositis and duodenal papillary carcinoma

Ning Wang
Department of Medical Examination Center Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou 310006, P.R. China

Objective: To investigate the CT findings of chronic duodenal papilla mucositis and duodenal papillary carcinoma, and provide more imaging information for early diagnosis of duodenal malignant diseases. **Methods**: CT findings and clinical data of 40 patients with chronic duodenal papilla mucositis and46 patients with duodenal papillary carcinoma were retrospectively analyzed. Observation and measuring of direct duodenal papilla signs (including size, shape, density, enhancement uniformity, etc.), indirect duodenal papilla signs (including pancreaticobiliary dilatation) and clinical indicators (including tumor markers CA19-9, CA125, CEA, blood routine white blood cell count, bilirubin, age, gender, etc.) were carried out according to CT as well as statistical analysis. **Results**: There were significant differences in duodenal papilla regular morphology, age and CA19-9 ($P < 0.05$), and significant differences in duodenal papilla maximum transverse diameter, diameter of common bile duct, diameter of pancreatic duct, total bilirubin, direct bilirubin, and jaundice in duodenal papillary carcinoma group ($P < 0.01$). There were no significant differences in duodenal papilla enhancement uniformity, plain CT value, arterial CT value, portal CT value, enhancement uniformity, presence or not of calculus at the lower end, gender, CEA, CA125, white blood cell count, and abdominal pain with fever (all $P > 0.05$). **Conclusion**: CT is helpful for the diagnosis of duodenal papilla disease, but the CT findings of patients with duodenal papillary carcinoma tend to be similar to findings of chronic duodenal papilla mucositis, which is easy to lead to misdiagnosis, so comprehensive diagnosis should be made according to the direct and indirect CT signs as well as laboratory and clinical manifestations of duodenal papilla, so as to improve the diagnosis of duodenal papillary carcinoma, and reduce missed diagnosis and misdiagnosis.

Keywords: Chronic duodenal papilla mucositis; Duodenal papillary carcinoma; Early diagnosis; CT

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Therapeutic efficacy of sorafenib and plant-derived phytochemicals in human colorectal cancer cells

Mohamed-Salah Abaza I[1], Abdul-Majeed Bahman[1], Sarah Khoushaish[1], and Rajaa J. Al-Attiyah[2]
[1]Molecular Biology Program, Department of Biological Sciences, Faculty of Science, Kuwait University, P.O. Box 5969, Safat 13060, Kuwait; [2]Department of Microbiology and Immunology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait

The present study aimed to investigate the sequence-dependent anticancer effects of combined treatment with sorafenib (Sora), a Food and Drug Administration-approved multikinase inhibitor drug, and plant-derived phytochemicals (PPCs) on human colorectal cancer (CRC) cell growth, and proteins associated with the control of cell cycle and apoptosis. The cytotoxic effects of 14 PPCs on CRL1554 fibroblast cells were determined using an MTT assay. Moreover, the cytotoxicity of Sora, PPCs, and a combination of both on CRC cells were also investigated. Cell cycle analysis was performed using flow cytometry, and cell apoptosis was investigated using DNA fragmentation, Annexin V/propidium iodide double staining, and mitochondrial membrane potential analyses. The cell cycle- and apoptosis-associated protein expression levels were analysed using western blotting. Based on their low levels of cytotoxicity in CRL1554 cells at ≤20%, curcumin, quercetin, kaempferol, and resveratrol were selected for use in subsequent experiments. The combined treatment of sora and PPCs caused levels of CRC cytotoxicity in a dose-, cell type-, and schedule-dependent manner. Moreover, the combined treatment of CRC cells arrested cell growth at the S and G2/M phases, induced apoptotic cell death, caused extensive mitochondrial membrane damage, and altered the expression of the cell cycle and apoptotic proteins. Results of the present study highlighted a difference in the level of sora efficacy in CRC cells when combined with PPCs. Further in vivo and clinical studies using the combined treatment of sora and PPCs are required to determine their potential as a novel therapeutic strategy for CRCs.

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The prognostic value of LAYN in HPV-related head and neck squamous cell carcinoma and its Influence on immune cell infiltration

Chenjing Zhu
Department of Radiation Oncology, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & Affiliated Cancer Hospital of Nanjing Medical University, 42 Baiziting, Nanjing 210009, Jiangsu, P.R. China
Email: drchenjingzhu@gmail.com

Background: HPV-positive head and neck squamous cell carcinoma (HNSCC) exhibits different characteristics from HPV-negative tumors in terms of tumor development, clinical features, treatment response, and prognosis. Layilin (LAYN), which contains homology with C-type lectins, plays a critical role in tumorigenesis and cancer progression. However, the prognostic value of LAYN and the relationship between LAYN and immune infiltration levels in HPV-related HNSCC patients still require a comprehensive understanding. Herein, we aimed to assess the prognostic value of LAYN and to investigate its underlying immunological function in HPV-related HNSCC.
Methods: Through various bioinformatics methods, we analyzed the data from The Cancer Genome Atlas (TCGA), Tumor Immune Estimation Resource (TIMER) and Gene Expression Profiling Interactive Analysis (GEPIA) databases to explore the potential underlying oncogenic impression of LAYN, including the relevance of LAYN to survival outcomes, clinicopathological factors, immune cell infiltration, and immune marker sets in HPV-related HNSCC. The expression levels of LAYN and HPV were also verified in HNSCC patient tissues.
Results: LAYN was differentially expressed in a variety of tumors. The expression of LAYN in HNSCC was significantly higher than that in adjacent normal tissues (P<0.0001), and high expression of LAYN was correlated with poor overall survival (OS) in HNSCC patients (Hazard Ratio (HR) = 1.3, P=0.035). Moreover, LAYN expression level in HPV-positive HNSCC patients was significantly lower than that in HPV-negative patients, with HPV-positive HNSCC patients displaying a trend of favorable prognosis. In addition, the relationship between LAYN expression and immune infiltration levels in HPV-positive HNSCC group was less tightly correlated than that in HPV-negative HNSCC group, and there was a strong relationship between LAYN expression and markers of M2 macrophage (P<0.001) and exhausted T cells (P<0.05) in HPV-negative HNSCC. Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis suggested that LAYN potentially influenced tumor progression through HPV infection and other cancer-related pathways.
Conclusions: LAYN might contribute to tumorigenesis via its positive correlation with immune checkpoint molecules and tumor-associated macrophages (TAMs). Our study might provide a novel prognostic biomarker and latent therapeutic target for the treatment of HPV-related HNSCC.

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Development of novel miR-dependent genome-editing adeno-associated virus that selectively eradicates glioblastoma-initiating cells

Toru Kondo
Division of Stem Cell Biology, Institute for Genetic Medicine, Hokkaido University, Japan

Glioblastoma (GBM), one of the most malignant human cancers, frequently recurs despite multimodal treatment with surgery and chemo/radiotherapies. One of the reasons of why GBM recues is likely the existence of GBM-initiating cells (GICs) that have strong proliferative and tumorigenic abilities and are resistant to various types of chemotherapies and radiotherapy. It is therefore crucial to find novel methods that specifically kill GICs by targeting their characteristics. Previously, we have identified various factors, such as membrane proteins, transcription factors and microRNA (miR), which increase or decrease in GICs compared with normal neural stem cells (NSC), and demonstrated their functions in GICs. On the process developing novel methods for GBM therapy, we noticed that these factors are considerably expressed in the cells of noncentral nervous system, suggesting the concerned side effects if we target these factors for therapy. To overcome this hurdle, we combined our previous findings with the genome-editing system and developed new miR-dependent genome-editing Adenoassociated virus (AAV) that selectively killed GICs without the off-target effects to normal cells. I will present the anti-tumorigenic ability of our new AAV in my talk.

Key words: glioblastoma, GIC, miR, genome-editing, AAV

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Changes in mitochondrial morphology and positioning in cancer cell death caused by mitochondrial oxidative stress

Yoshihiro Suzuki-Karasaki and Manami Suzuki-Karasaki
Plasma ChemiBio Laboratory, Nasushiobara, Tochigi, Japan
E-mail: suzuki.pcbl@gmail.com

Mitochondria are highly plastic, dynamic, and heterogenous organelles that control diverse functions. An emerging view is that mitochondrial shape and location changes are active events coupled with cell functions, death, and survival. Due to unique metabolic traits, cancer cells are more susceptible than nontransformed cells to mitochondrial oxidative stress (mitoOS) caused by chemical and physical interference, such as chemicals, oxidants, and cold atmospheric plasmas. We recently reported that mitoOS resulted in drastic changes in mitochondrial shape and positioning in cancer cells (Suzuki-Karasaki M et al. *Int J Mol Sci* 2022). Upon mitoOS, cancer cell mitochondria lost the reticular network structure, underwent irreversible fragmentation, and assembled the juxtaposition of the damaged site of nuclei (called monopolar perinuclear mitochondrial clustering, MPMC hereafter). MPMC is cytodestructive and distinct from perinuclear mitochondrial clustering (PNMC), which can protect cancers from hypoxia by facilitating hypoxia-induced factor signaling. In contrast, only modest, maybe reversible fragmentation was observed in nontransformed cells. MPMC may involve three processes: mitochondrial fragmentation by H2O2 or nitric oxide, transport along the microtubule, and plasma membrane depolarization-mediated assembly. Moreover, other organelles, including endoplasmic reticulum were also found to be involved in cell death. Finally, in support of the role of MPMC in nuclear injury, the damaged nuclei had increased oxidative stress.

Reference

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From liquid biopsy to microRNA therapeutics for colorectal cancer (CRC)

Jessica Gasparello[1], Matteo Zurlo[1], Chiara Papi[1], Patrizio Giacomini[2], Roberto Corradini[3], Roberto Gambari[1] and Alessia Finotti[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [2]Oncogenomics and Epigenetics, IRCCS (Istituto di Ricovero e Cura a Carattere Scientifico) Regina Elena National Cancer Institute, Roma, Italy; [3]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy
E-mail: alessia.finotti@unife.it

Liquid biopsy (LB) provides an examination of the peripheral blood from cancer patients for circulating tumor cells, cell-free nucleic acids and microRNAs (miRNAs) and is an established tool for precision medicine. The miRNA profile in plasma samples isolated from a cohort of 35 CRC patients, at the day of surgery, was analyzed by Next Generation Sequencing (NGS) and further confirmed by droplet digital RT-PCR (dd-RT-PCR). NGS-based miRNome analysis of a training cohort of five CRC and three tumor-free donors identified a novel, distinct nine miRNA signature comprising five up-regulated and four down-regulated miRNAs, six of which have been confirmed in the full CRC and tumor-free donor validation dataset by dd-RT-PCR. The miRNA list provides diagnostic markers as well as possible molecular targets for protocols focusing on "microRNA therapeutics". The biological effects resulting from the targeting of the most relevant dysregulated miRNAs of the nine-miRNA signature with anti-miRNA peptide nucleic acids (PNAs) were verified, and their anti-cancer activity in terms of apoptosis induction was evaluated. Our data demonstrate that targeting bloodstream up-regulated miRNAs using anti-miRNA PNAs leads to the down-regulation of target miRNAs associated with the activation of the pro-apoptotic pathway in CRC cellular models. For example, the microRNAs miR-15b-5p, miR-584-5p and miR-425-3p are up-regulated in plasma of colorectal cancer (CRC) patients: targeting with inhibitor peptide nucleic acids is associated with induction of apoptosis in colon cancer cell lines (HT29 and LoVo). Moreover, very high percentages of apoptotic cells were found when the anti-miRNA PNAs were associated with other pro-apoptotic agents, such as sulforaphane (SFN). The presented data sustain the idea that the targeting of miRNAs up-regulated in the bloodstream with a known role in tumor pathology might be a tool for the design of protocols for anti-tumor therapy based on miRNA-targeting molecules.

This work was supported by the European Union (EU) Horizon 2020 Research and Innovation Programme (GA #633937, project ULTRAsensitive PLAsmonic devices for early CAncer Diagnosis (ULTRAPLACAD), by the Interuniversity Consortium for Biotechnology, Italy and by the FIR 2020 and FAR 2022 funds from the University of Ferrara to A.F. J.G. is funded by the FIRC-AIRC "Michele e Carlo Ardizzone" fellowship.

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Genomic instability in adenomas and in colorectal cancer progression

Pavel Vodička[1,2,3], Michal Kroupa[1,3] Kristyna Tomasova[1,3], Anna Siskova[1,2], Katerina Balounova[1], Sona Vodenkova[1,3], Veronika Vymetalkova[1,2,3], Rajiv Kumar[1], Safaa Andarawi[1], Saba Selvi[1], Stefanie Brezina[4], Andrea Gsur[4], Kari Hemminki[3] and Ludmila Vodickova[1,2,3]
[1]Institute of Experimental Medicine, ASCR Prague; [2]Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague; [3]Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic; [4]Institute of Cancer Research, Department of Medicine I, Medical University Vienna, Vienna, Austria
E-mail: pavel.vodicka@iem.cas.cz

In solid cancers both impaired DNA repair and disrupted telomere length (TL) homeostasis are key culprits in cancer initiation, progression and prognosis. Telomere attrition resulting in replicative senescence, simultaneously by-passing cell cycle checkpoints, is a hallmark of cellular malignant transformation. Telomerase, ubiquitous in advanced solid cancers, is fundamental to cell immortalisation. Human solid neoplasms often exhibit chromosomal instability (CIN), which generates either abnormal aneuploid karyotypes, or continually expands phenotypic heterogeneity as tumor cell populations undergoing cell divisions. We searched for the CIN markers in the adenoma-adenocarcinoma transition and in CRC progression. Understanding the mechanisms and dynamics of tumor genomic diversification, where DNA damage response and telomere homeostasis are important players, is critical in understanding carcinogenesis and overcoming drug resistance. A part of the above search is the comparison of telomere homeostasis genetics (based on GWAS study) with TL in 7,000 patients with sporadic CRC. The mitochondrial dysfunction, another cancer hallmark, is linked with DNA repair capacity and compensate for damage by increasing the mitochondrial DNA copy number (mtDNA-CN). Current studies on the mtDNA-CN reported ambiguous and inconsistent results for various cancer types. Telomere shortening has a dual role in tumorigenesis. It promotes cancer initiation by inducing CIN, while TL maintenance characterized by telomerase expression is required for cancer cell proliferation and tumour growth. The reports on TL as a biomarker for cancer risk, patient therapy response and/or survival are contradictory as well. Our investigations were also focused on mtDNA_CN in CRC tissues and adjacent mucosa.

Acknowledgement: NU21-03-00145, NU21-07-00247, 21-27902S, 21-04607X. The project National Institute for Cancer Research (LX22NPO5102).

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H3K27me3-mediated inactivation of SFRP1 promotes cell proliferation via Wnt/β-Catenin signaling pathway in esophageal squamous cell carcinoma

Menghan Zhou[1#], Shenling Yu[1#], Ying Xu[2], Min Liu[2], Yanping Ge[2], Hong Fan[1*]
[1]Department of Medical Genetics and Developmental Biology, Medical School of Southeast University, The Key Laboratory of Developmental Genes and Human Diseases, Ministry of Education, Southeast University, Nanjing, P.R. China; [2]School of Life Science and Technology, Southeast University, Nanjing, P.R. China

To investigate the effects of H3K27me3-mediated inaction of tumor suppressor genes and its function in esophageal squamous cell carcinoma (ESCC), H3K27me3-enriched genomic DNA fragments in ESCC cell lines EC9706 was detected by ChIP-seq and indicated H3K27me3 was widely distributed in the genome of ESCC cells. SFRP1 was identified as a candidate gene regulated by H3K27me3 because H3K27me3 deposited on the upstream region of SFRP1 promoter and inactived SFRP1 expression. Furthermore, we found SFRP1 was significantly down-regulated in ESCC tissues compared with corresponding adjacent non-tumor tissues, and the expression level of SFRP1 was significantly associated with TNM stage and lymph node metastasis. Over-expression of SFRP1 significantly suppressed cell proliferation in ESCC. Over-expression of SFRP1 was also found to be negatively correlated with the expression of β-Catenin in the nucleus, as showed by decreased expression of c-Myc and CyclinD1 through deactivation of the Wnt/β-Catenin signaling pathway. The present findings are the first to reveal that H3K27me3-mediated SFRP1 inhibit the proliferation of ESCC cells by participating in deactivation of the Wnt/β-Catenin signaling pathway.

Key words: ESCC; H3K27me3; SFRP1; Wnt/β-Catenin signaling pathway; Cell proliferation

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HPV16 E6 gene transcripts in primary type II endometrial carcinomas

Wiktor Szewczuk[1], Oksana Szewczuk[2], Krzysztof Czajkowski[2], Maciej Wałędziak[3], Barbara Górnicka[4], Tomasz Ilczuk[4], Weronika Kawecka[5], Andrzej Semczuk[5]
[1]Department of Pathology, Military Institute of Medicine, Warsaw, Poland; [2]II[nd] Department of Obstetrics and Gynecology, Medical University, Princess Anna Mazowiecka Hospital, Warsaw, Poland; [3]Department of Surgery, Military Institute of Medicine, Warsaw, Poland; [4]Department of Pathology, Medical University, Warsaw, Poland; [5]II[nd] Department of Gynecology, Lublin Medical University, Lublin, Poland

Data are limited on the prevalence of human papilloma virus (HPV) DNA in different subtypes of endometrial carcinomas (EC). We investigated the incidence of HPV16 DNA E6/E7 transcripts in 47 type I (endometrioid-type) tumors and eight type II (non-endometrioid-type) uterine neoplasms applying PCR-based technology. Immunohistochemical staining in HPV16 positive cases was also performed, and seven lymph node metastases were examined for the prevalence of HPV16 DNA E6/E7. None of the type I ECs displayed HPV16 E6 gene transcripts; however, four out of 8 (50%) type II ECs (two out of four papillary-serous and two out of four clear-cell carcinomas) contained HPV16 E6 transcripts. The difference HPV16 E6 gene transcripts positivity between endometrioid and non-endometrioid neoplasms was statistically significant (p=0.0011). Apart from the cancer subtype, none of the EC clinicopathological features were related to HPV16 E6 positivity. None of 55 ECs contained an HPV16 E7 gene transcripts. Immunohistochemistry confirmed HPV16 positivity with all slides from gene-positive samples, showing intense staining reactions. Interestingly, virus was not detected in any of seven lymph node metastases, including four from HPV16-positive primary tumors. HPV16 E6 gene transcripts may be detected in ECs, primarily in the non-endometrioid (type II) uterine cancer subtypes. HPV16 E6/E7 transcripts are not found in lymph node metastases, even when primary tumors were virus-positive.

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Hereditary colorectal cancer: State of the art in Lynch syndrome

Francesca Duraturo[1], Marina De Rosa[1], Paola Izzo[1]
[1]Department of Molecular Medicine and Medical Biothecnologies, University of Napoli, Federico II, Italy
E-mail: francesca.duraturo@unina.it

Hereditary non-polyposis colorectal cancer is also known as Lynch syndrome. Lynch syndrome is associated with pathogenetic variants in one of the mismatch repair (MMR) genes. In addition to colorectal cancer, the inefficiency of the MMR system leads to a greater predisposition to cancer of the endometrium and other cancers of the abdominal sphere. Molecular diagnosis is performed to identify pathogenetic variants in MMR genes. However, for many patients with clinically suspected Lynch syndrome, it is not possible to identify a pathogenic variant in MMR genes. Molecular diagnosis is essential for referring patients to specific surveillance to prevent the development of tumors related to Lynch syndrome. This review summarizes the main aspects of Lynch syndrome and recent advances in the field and, in particular, emphasizes the factors that can lead to the loss of expression of MMR genes.

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METTL18 is a novel phenotypic regulator of Src-dependent metastatic response of HER2-negative breast cancer

Han Gyung Kim, Ji Hye Kim, Jae Youl Cho
Department of Integrative Biotechnology, Sungkyunkwan University, Suwon 16419, Republic of Korea

Methyltransferase-like (METTL)18 has histidine methyltransferase activity on the RPL3 protein and is involved in ribosome biosynthesis and translation elongations. Several studies have reported that actin polymerization serves as a Src regulator, and HSP90 is involved in forming polymerized actin bundles. To understand the role of METTL18 in breast cancer and the metastasis of HER-2 negative breast cancer, we used biochemical, molecular biological, and immunological approaches *in vitro* (breast tumor cell lines), *in vivo* (tumor xenograft model), and in samples of human breast tumors. A gene expression comparison of 31 METTL series genes and 22 methyltransferases in breast cancer patients revealed that METTL18 is highly amplified in human HER2-negative breast cancer. In addition, in HER2-negative breast cancer patients, a high expression of METTL18 leads to a poor prognosis. Reducing of METTL18 significantly inhibited the activity metastasis of breast cancer cells *in vivo* and *in vitro*. METTL18 indirectly regulates the phosphorylation of the proto-oncogene tyrosine-protein kinase Src and its downstream molecules in MDA-MB-231 cells via METTL18-mediated RPL3 methylation, which is also involved in determining HSP90 integrity and protein levels. In confocal microscopy and F/G-actin assays, METTL18 was found to induce actin polymerization via HSP90. Interestingly, molecular events involving METTL18, RPL3, HSP90, and actin polymerization yielded Src phosphorylated at both tyrosine 416 and tyrosine 527 with kinase activity and oncogenic functions. Therefore, the METTL18-HSP90-Actin-Src regulatory axis plays critical oncogenic roles in the metastatic responses of HER2-negative breast cancer and could be a promising therapeutic target.

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Assessment of preanalytical conditions of prostate markers

Michal Jirásko[1,3], Radek Kučera[1,3], Radana Vrzáková[2], Václav Šimánek[1,3], Roman Viták[3], Ondřej Topolčan[1]
[1]Department of Immunochemistry Diagnostics, University Hospital and Faculty of Medicine in Pilsen; [2]Department of Medical Chemistry and Biochemistry, Faculty of Medicine in Pilsen, Charles University; [3]Department of Pharmacology and Toxicology, Faculty of Medicine in Pilsen, Charles University, Czech Republic

Failure to adhere to preanalytical conditions can lead to innacurate prostate biomarker levels, burdening patients. To evaluate sample stability, we adhered to the International Society for Biological and Environmental Repositories (ISBER) recommended standard operating procedure (SOP), which outlines the criteria for assessing biomarker stability post-separation, during storage and refreezing. The study's objective was to determine the ideal preanalytical conditions for tPSA, fPSA, and -2proPSA. Blood samples were obtained from 45 volunteers with tPSA concentrations within the range of 2-10 µg/L with serum separated within an hour. Subsequently, these samples were subjected to various stressors as per a modified ISBER-recommended protocol, followed by immediate freezing at -80°C for subsequent storage and processing. The quantification of tPSA, fPSA, and -2proPSA levels was accomplished using the chemiluminescence method with a UniCel DxI 800 analyzer (Beckman Coulter, USA). Following this, the calculation of the fPSA to tPSA ratio and Prostate Health Index (PHI) was performed, and statistical analysis of the resultant data ensued. Changes in biomarker levels following exposure to freeze-thaw cycles exhibited statistical significance in some instances but remained clinically insignificant. Our study revealed that tPSA exhibited remarkable stability when stored at both examined temperatures. Conversely, the stability of fPSA showed a gradual decline over time at both temperatures, with the most pronounced decrease observed at the 72h mark at room temperature, which holds clinical relevance. This phenomenon led to a reduction in the fPSA to tPSA ratio, elevating the risk of false positive diagnoses. Additionally, a progressive increase in -2proPSA levels was observed at both temperatures, with a notably significant clinical impact observed at 72h at room temperature. The observed decline in fPSA and rise in -2proPSA had a profound influence on PHI, elevating the risk of false positive diagnoses. We demonstrated a high stability of tPSA under different preanalytical conditions. Furthermore, lower stability of fPSA and -2proPSA was demonstrated. Inappropriate transport and storage conditions may lead to false positive evaluation of laboratory results. This then leads to further unnecessary diagnostic burden on the patient or even an unnecessarily indicated prostate biopsy.

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MicroRNA-204 inhibits vasculogenic mimicry and angiogenesis in stem-like breast cancer cells

Martha Resendiz-Hernández[1], Estefania Contreras-Sanzón[1], Macrina B. SilvaCázares[2], Laurence A. Marchat[3], Olga N. Hernández de la Cruz[1], Mario César López-Camarillo[1]
[1]Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México, México; [2]Coordinación Academica Región Altiplano, Universidad Autónoma de San Luis Potosí. San Luis Potosí, México; [3]Programa en Biomedicina Molecular y Red de Biotecnología. Instituto Politécnico Nacional, México. Email: cesar.lopez@uacm.edu.mx

Angiogenesis and vasculogenic mimicry are two cancer hallmarks essential for cancer cells survival. Both mechanisms coexist in aggressive neoplasia and feed the cells to fuel tumor growth. Vasculogenic mimicry is characterized by formation of three-dimensional (3D) channels-like structures by tumor cells, supplying the nutrients needed for tumor growth. Angiogenesis and vasculogenic mimicry are both stimulated by hypoxic tumor microenvironment which activates common signaling pathways, and they have been associated with increased metastasis and clinical poor outcome in breast cancer patients. Recent reports, indicate that microRNAs may regulate genes involved in both cancer hallmarks, but the molecular mechanism remains to be fully elucidated. We aim to study the functional relationships between angiogenesis, vasculogenic mimicry and microRNA-204-5p (miR-204) tumor suppressor in cancer stem-like cells (CSCs) isolated from MDA-MB-231 breast cancer cell lines (immunophenotype CD44+/CD24-). Data showed that miR-204 restoration using RNA mimics in CSCs significantly reduce the number and size of CSCs spheroids. Moreover, miR-204 restoration in CSCs leads to a potent inhibition of vasculogenic mimicry which was associated to a reduction of number of branch points and patterned 3D like-channels. In addition, miR-204 mimics also significantly inhibited angiogenesis *in vitro*. The number of tubules and nodes was significantly reduced in CSCs transfected with miR-204 mimics. Further analysis of activation state of signaling pathways revealed that miR-204 reduced the expression and phosphorylation of AKT1 in CSCs. In conclusion, our study provides novel lines of evidence indicating that miR-204 may exerts a finetunning regulation of the transduction of AKT1 a novel mediator critical for vasculogenic mimicry formation and angiogenesis in breast cancer stem-like cells. These data highlight the potential utilization of miR-204 as a novel therapeutic tool in breast cancer.

Acknowledgments. We thanks to Universidad Autónoma de la Ciudad de México for assistance support. This research was funded by Consejo Nacional de Humanidades, Ciencias y Tecnologias, CONAHCYT Mexico (grant number A1S-13656).

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Proteoglycans and glycosaminoglycans contribute to high heterogeneity and chemoresistance of glioblastoma cells

Sofia A. Nikitina[1], Dmitry K. Sokolov[1], Alexandra Y. Tsidulko[1], Anastasia V. Strokotova[1], Elizaveta Fasler-Kan[2], Elvira V. Grigorieva[1,3*]
[1]Institute of Molecular Biology and Biophysics FRC FTM, Russia; [2]Department of Pediatric Surgery Children's Hospital, Inselspital Bern, University of Bern, 3012 Bern, Switzerland; [3]Novosibirsk State University, Novosibirsk, Russia
E-mail: elv_grig@yahoo.com

Resistance of cancer cells to chemotherapy is one of the main reasons of relapse development and low patients survival. Although numerous molecular mechanisms of chemoresistance are studied, a role of glycosylated macromolecules in this process remain underinvestigated, especially for glioblastoma (GB). In this study, seven human GB cell lines were characterized for temozolomide (TMZ) sensitivity, cell phenotypic traits, expression levels of PG core proteins- and heparan sulfate (HS) biosynthesis-related genes and content of their chondroitin sulfate (CS) and HS chains. It was shown that inspite of the similar proliferation rates, the cell lines posessed different migration activity, clonogenicity, TMZ resistance, cell-line specific expression of some PGs (NG2/CSPG4, CSPG5, versican) and GAG content (CS/HS). Among the cell lines, LN18 cells were the most resistant to TMZ and had 2.5-fold higher PG core proteins expression than that in U343 most sensitive cells, whereas overall transcriptional activity of HS biosynthesis-involved genes was low in all GB cell lines. Mainly EXT1/2 and NDST1/2 (responsible for HS chains elongation) were expressed in the studied cells, while expression of sulfotransferases and sulfatase2 were cell line-specific, suggesting different pattern of sulfation of the HS chains in GB cells. Taken together, these data demonstrate a contribution of PGs/GAGs into high heterogeneity and TMZ resistance of GB cells suggesting them as potential biomarkers of TMZ resistance.

The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No.122032200240-8, code FGMU-2022-0005). AVS was supported by a Scholarship of Russian Federation President for young scientists (SP-4000.2022.4).

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Exploring novel ligands as potential inhibitors and substrates for bovine serum amine oxidase: Biochemical insights and molecular modeling studies

Rino Ragno[1], Eleonora Proia[1], Alessio Ragno[2] and Enzo Agostinelli[3,4]
[1]Rome Center for Molecular Design, Department of Drug Chemistry and Technology, Sapienza Università di Roma, P. le A. Moro 5, 00185 Roma, Italy, rino.ragno@uniroma1.it (R.R.); eleonora.proia@uniroma1.it (E.P.)
[2]Department of Computer, Control, and Management Engineering "Antonio Ruberti", Sapienza University, 00185 Rome, Italy. alessio.ragno@uniroma1.it (A.R.)
[3]Department of Sensory Organs, Sapienza University of Rome, Policlinico Umberto I, Viale del Policlinico155, I-00161 Rome, Italy, enzo.agostinelli@uniroma1.it (E.A.)
[4]International Polyamines Foundation 'ETS-ONLUS' Via del Forte Tiburtino 98, I-00159 Rome, Italy, polyfoundation2017@gmail.com

Bovine Serum Amine Oxidase (BSAO) is a critical enzyme involved in the oxidative deamination of various biogenic amines such as spermine and spermidine, playing a pivotal role in regulating physiological processes. In this study, we present a series of novel ligands as a potential inhibitor and substrate for BSAO. The ligand were selected through a previous virtual screening and based on a rational drug design approach. It was observed that they incorporated structural features for potential binding with the enzyme's active site. Biochemical assays were conducted to evaluate the inhibitory activity of the ligand against BSAO. The obtained data revealed a notable dose-dependent inhibition with an impressive IC_{50} values, indicating a strong potential for the ligands as effective inhibitors. Additionally, kinetic studies were performed to elucidate the mode of inhibition and provided valuable insights into the ligand-enzyme interaction. Furthermore, molecular modeling techniques by means of the www.3d-qsar.com portal were employed to elucidate the binding mechanism and to gain a detailed understanding of the ligand-enzyme complex. Docking studies demonstrated favorable interactions between the ligand and key residues within the active site of BSAO, highlighting critical molecular determinants for inhibitory activity. The structural insights obtained from the molecular modeling studies will guide further optimization of the ligand, aiming to enhance its potency and selectivity towards BSAO. Additionally, this study provides a valuable foundation for future investigations into the development of novel therapeutic agents targeting BSAO, with potential applications in various physiological and pathological contexts.

Keywords: Bovine Serum Amine Oxidase, inhibitor, substrate, ligand design, biochemical assays, molecular modeling, docking, molecular dynamics simulations.

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rDNA genes, development and cancer

Nickolai A. Tchurikov
Engelhardt Institute of Molecular Biology Russian Academy of Sciences, Moscow, Russia

The accumulated data suggest a link between expression of rDNA clusters and differentiation. In early development the formation of epigenetically silenced rDNA is associated with the formation of condensed chromatin structures outside of the nucleolus, as well as transcriptional activation of a set of differentiation genes. One possible underlying mechanism is the involvement of nucleoli in the global epigenetic regulation of thousands developmental genes via formation of direct inter-chromosomal contacts between rDNA clusters and different chromosomal regions that are involved in formation of either active or repressed condensates. We used the 4C (circular chromosome conformation capture) approach to determine the whole-genome contacts of rDNA clusters (4C-rDNA) in HEK293T cells which were originally isolated from human embryonic kidney. The numerous inter-chromosomal contacts were observed with different genomic regions that possessed in the close neighborhood (about 2.5 kb) hundreds of genes controlling nervous system development and cell morphogenesis. Among rDNAcontacting genes there were both active and silent genes. Among the latter's been *DUX4* genes residing at the tip of chr4. Numerous rDNA contacts were also detected at the chromosomal regions possessing very long (from 5 to 50 kb) stretches of the H3K27ac mark which is associated with super-enhancers. Gene ontology searches suggest that rDNA-contacting genes are involved in development and morphogenesis. About 100 of these genes in different human cell lined are highly associated with silencing by the H3K27me3 mark in several normal cell types, including bronchial epithelial cells, keratinocytes, myoblasts, monocytes, endothelial cells, and kidney epithelial cells. Thus, a concerted silencing of a specific group of rDNA-contacting genes controlling development occurs during differentiation. Detailed analysis of rDNAcontacting genes revealed that they are highly associated with different cancers: skin cancer, melanoma, endometrial cancer, breast, lung, and liver cancers.

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Comparison of specific myo/fibroblast markers in fibroblasts isolated from various neoplastic/healing/normal tissues

Lukáš Urban[1,2]
[1]Department of Pharmacology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic
[2]Department for Biomedical Research, East-Slovak Institute of Cardiovascular Diseases, Inc., Košice, Slovak Republic
E-mail: lurban@vusch.sk

Fibroblasts are a diverse population of mesenchymal cells that contribute to the maintenance and production of connective tissue. They play a crucial role in deposition and remodelling of extracellular matrix (ECM) through the production of structural macromolecules and proteolytic enzymes, which can result in aberrant fibrosis when activated inappropriately. Fibroblasts may differentiate into myofibroblasts, a highly contractile α-smooth muscle actin+ (SMA+) phenotype, and become critical for wound contraction. Cancer-associated fibroblasts (CAFs) resemble myofibroblast-like cells and have been shown to modulate the biological properties of tumors. In addition to their role in ECM production, fibroblasts also create a signaling niche by producing various cytokines/chemokines, growth factors, and other signaling molecules, which regulate clinically relevant parameters of the tumor/wound microenvironment (TME/WME), depending on their origin and acquired phenotype. Considering the similarities between wound healing and tumor progression, we investigated the expression of specific myo/fibroblast markers in fibroblasts isolated from various neoplastic, healing, and normal tissues. Our focus was on identifying markers with reported biological implications in fibrosis, including prognostic value.

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Stroma-derived stimuli in FGFR2-mediated signalling in luminal breast cancer: A missing link between in vitro and in vivo findings?

Hanna M. Romanska[1], Marcin Braun[1], Julia Solek[1], Lukasz Turczyk[2], Dominika Piasecka[2], Rafal Sadej[2]
[1]Department of Pathology, Medical University of Lodz; [2]Department of Molecular Enzymology and Oncology, Intercollegiate Faculty of Biotechnology, Medical University of Gdansk, Poland
E-mail: hanna.romanska@gmail.com

Fibroblast Growth Factor (FGF) Receptor (FGFR)-mediated interactions between tumour microenvironment (TME) and breast cancer (BC) cells have long been implicated in the progression and response to therapy. Using an *in vitro* model (MCF7 and T47D, luminal BC cell lines and their FGFR2-deficient mutants, $MCF^{/FGFR2-}$ and $T47D^{FGFR2-}$) we have demonstrated that: i) signalling mediated by FGFR2 caused ER phosphorylation, ubiquitination and subsequent ER proteasomal degradation, which counteracted tamoxifen-promoted ER stabilization[1]; ii) FGF7 stimulated activation of PI3K/AKT, leading to phosphorylation of ER at Ser167 and upregulation of Bcl-2, both of which mediated FGF7/FGFR2-driven resistance to the drug[1]; iii) FGF7/FGFR2- triggered signalling induced phosphorylation of PR at Ser294 through RSK2, which resulted in receptor ubiquitination and subsequent degradation via the 26S proteasome pathway[2]. Although the findings are consistent with available data from other preclinical studies, a clinical evidence supporting a protumorigenic role of FGFR in BC (including clinical trials with FGFR inhibitors) is still missing. Here, analysis of data of BC patients (N=353) using immunohistochemistry and Nanostring-based RNA quantification demonstrated that the prognostic value of FGFR2 varied between patients with different menopausal status. Low FGFR2 was associated with higher grade, higher Ki67 proliferation index ($p<0.001$) and worse overall and disease-free survival (HR=2.34 (95% CI: 1.26-4.34), p=0.007 and HR=2.22 (95% CI: 1.25-3.93), p=0.006, respectively). The poor prognostic value of low FGFR2 was apparent in ER+PR+, but not in ER+PR- patients, and it did not depend on the expression level of PR-dependent genes[3]. This suggests that the role of FGFR2 in BC is more complex and that the stromal component, that activates or represses its function, may represent a 'missing link' in the translation between mechanistic studies and clinical evaluation of its true prognostic and predictive value.

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Acknowledgements: Research supported by the National Science Centre Grant 2020/37/B/NZ4/01900.

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CRISPR/Cas9 screens to define the membrane transporters at the metabolic intersection with hypusination of eIF5A

Yee Kwan Law[1], Patrick Essletzbichler[1], Giulio Superti-Furga[1,2]
[1]CeMM Research Centre for Molecular Medicine of the Austrian Academy of Sciences, Vienna; [2] Medical University of Vienna, Vienna, Austria
Email: ylaw@cemm.oeaw.ac.at

Hypusination of the highly conserved and abundant protein eIF5A depends on the conversion of the polyamine spermidine via successive catalytic action of deoxyhypusine synthase (DHPS) and deoxyhypusine hydroxylase (DOHH). eIF5A exerts translational control over polyproline motif-containing mRNAs by binding to the E-site of the ribosome, requires hypusination for activity and regulates cell growth and mitochondrial metabolism (Puleston et al. 2019 Mol Cell). Therefore, polyamine synthesis and import are linked to growth and bioenergetics via hypusination as linkpin. We have functionalized a monoclonal antibody and configured a FACS-based selectable screen. We also have constructed a library of guides specifically targeting membrane transporters of the solute carrier supergroup, ABC transporters, P-type ATPases and aquaporins. Preliminary screens show that indeed it is possible to identify transporter genes that affect eIF5A hypusination. I plan to perform various screens to obtain a robust list of hits. We will validate the screen hits by generating individual KO or over-expression cell lines, and to elucidate the mechanism through which membrane transporters regulate hypusine by using metabolomic and proteomic methods as well as genetic epistatic analysis and chemical treatments. Eventually, we expect these findings to reveal a therapeutically tractable modality through which membrane transporters can regulate eIF5A hypusination and mitochondrial function.

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A new mechanism of oxidative DNA damage by a putative acrylamide metabolite, acrylohydroxamic acid

Yurie Mori, Hatasu Kobayashi, Mariko Murata, Shinji Oikawa
Department of Environmental and Molecular medicine, Mie University Graduate School of Medicine, Edobashi 2-174, Tsu, Mie 514-8507, Japan
E-mail: m-yurie@med.mie-u.ac.jp

Acrylamide (ACR) is widely used for soil stabilization, water treatment, and in industrial product manufacturing. ACR is formed in carbohydrate-rich foods (e.g., potatoes and cereals) heated at high temperatures, as a result of Maillard reactions. ACR is found in cigarette smoke, and this may be one of the main routes of ACR exposure in smokers. There is sufficient evidence that ACR is carcinogenic in rodents, and the International Agency for Research on Cancer (IARC) classifies ACR as a probable human carcinogen (Group 2A). Glycidamide, an epoxide metabolite of acrylamide, is implicated in the mechanism of ACR carcinogenicity. On the other hand, oxidative DNA damage is observed in the liver tissues of rats treated with ACR. We sought to clarify the mechanism of ACR-induced oxidative DNA damage by investigating site-specific DNA damage and reactive oxygen species (ROS) generation by a putative metabolite of ACR, acrylohydroxamic acid (AA). Our experiments, using ^{32}P-5'-end-labeled DNA fragments, indicated that, amidase-treated AA induced DNA damage in the presence of Cu(II) although AA alone did not damage DNA. DNA cleavage occurred preferentially at T and C, and particularly at T in 5'-TG-3' sequences, and the DNA cleavage pattern was similar to that of hydroxylamine. The DNA damage was inhibited by methional, catalase, and Cu(I)-chelator bathocuproine, suggesting that the involvement of H_2O_2 and Cu(I) in the mechanism of DNA damage induced by amidase-treated AA. In addition, amidase-treated AA increased 8-oxo-7,8-dihydro-2'-deoxyguanosine formation in calf thymus DNA, an indicator of oxidative DNA damage, in a dose-dependent manner. In conclusion, hydroxylamine, a possible product from AA treated with amidase, was autoxidized via the Cu(II)/Cu(I) redox cycle and H_2O_2 generation, suggesting that oxidative DNA damage induced by ROS plays an important role in ACR-related carcinogenesis (1).

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Development of a rapid diagnostic test for detection of pathogenic mycobacteria based on CRISPR

Lia Lima, Marcia Quinhões, Sidra Vasconcellos, Raquel Teixeira Edson Machado, Bruna Petrilho, Victória Moraes, Carolyne Cristhine, Harrison Gomes, Philip Suffys
Laboratory of Molecular Biology applied to Mycobacteria -Fiocruz, Brazil
E-mail: lialimagomes@hotmail.com

Non-Tuberculous Mycobacteria (NTMs) are pathogens (opportunists) that can cause lung diseases with symptoms similar to tuberculosis (TB), in addition to other clinical manifestations. In recent years there has been a considerable increase in NTM lung diseases in Brazil (1,2). The high prevalence of TB in the country makes it difficult to correctly identify NTM, leading to inadequate diagnosis and treatment. It is reported that 58% of patients reported with NTM lung disease in Rio de Janeiro were initially reported and treated as TB (3). Current etiological methods used in microbiology laboratories are complex, expensive, time-consuming and have low sensitivity and specificity. In this work, we propose the development of a rapid diagnostic test based on CRISPR CAS12/CAS13 capable of differentiating the main NTM species (4), presenting high sensitivity and specificity for diagnosis (4) and enabling the monitoring of active disease, such as monitoring efficacy of the treatment. Cas12a is an RNA-guided endonuclease that can directly bind and cut specific target DNA (4). Cas13 is a nuclease that has specific recognition and cleavage activity for complementary RNA and has been used in in vitro mRNA detection procedures (5). Both enzymes are capable of performing collateral transcleavage, allowing the use of probes that signal the presence of target DNA or RNA (5,6,7). This methodology is innovative because it allows a specific diagnosis in a few hours and at a low cost. We managed to clone, express and purify the cas12a protein at LABMAM – FIOCRUZ. The RNAS guide was specific for the recognition of M. tuberculosis, M. kansasii, M. abscessus, M. leprae, M. avium. However, sensitivity and specificity testing is ongoing. In the future, we will seek partnerships to validate our tests in diagnostic kit format to adapt to different types of users and clinical demands of the Unified Health System.

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Financial support: Faperj and Inova fiocruz

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Antineoplastic and antiangiogenic effects of novel tyrosine kinase inhibitors in hepatocellular cancer cells

Andi Ma[1], Nils Göhringer[1], Bianca Nitzsche[1], Bernhard Biersack[2], Michael Höpfner[1]
[1]Institute of Physiology, Charité-Universitätsmedizin Berlin, Corporate Member of the Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Charitéplatz 1, 10117 Berlin, Germany; [2]Organic Chemistry Laboratory, University Bayreuth, Universitaetsstrasse 30, 95440 Bayreuth, Germany
E-mail: michael.hoepfner@charite.de

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer death worldwide. Especially for advanced and metastatic HCC effective treatment options are still lacking. Thus, we evaluated the antineoplastic and antiangiogenic effects of two novel 2-(thien-2-yl)-acrylonitrile derivatives (Thio-Iva and Thio-Dam) with multikinase inhibitory activity and characterized their mode of action in HCC cells. As evidenced by crystal violet staining and real-time cell growth measurements (iCelligence) Thio-Iva and Thio-Dam displayed pronounced antiproliferative effects in hepatocellular HUH-7 and SNU449 cancer cells with IC_{50} concentrations in the sub-micromolar or low micromolar range (Thio-Dam: 1.64±0.51 µM (SNU-449) and 0.81±0.26 µM (HUH-7) and Thio-Iva: 0.53±0.32 µM (SNU-449) or 0.29±0.26 µM (HUH-7). Cytotoxicity screenings excluded cytotoxicity as an unspecific mode of action of the novel compounds, while flow cytometry and Western Blot analyses revealed a dose-dependent and pronounced G2-M phase arrest and corresponding Cyclin B1 suppression of HCC cells. ROS-driven and mitochondria-related apoptosis with concomitant caspase-3 activation was also observed. The novel compounds effectively inhibited capillary tube formation of endothelial EA.hy926 cells *in vitro*, pointing towards additional antiangiogenic effects of Thio-Iva and Thio-Dam. Antiangiogenic and antineoplastic effects were confirmed in systemic measurements employing *in ovo* testing of HCC tumor bearing fertilized chicken eggs (CAM assays). Notably the novel compounds were even more powerful in reducing HCC tumor growth *in ovo* than the clinically relevant TK inhibitor sorafenib. In this study, we could demonstrate promising antineoplastic and antiangiogenic effects of two novel 2-(thien-2-yl)-acrylonitrile with multikinase inhibitory activity in HCC cells. The characterization of their mode of action as apoptosis- and cell cycle arrest-inducing agents render them interesting for further evaluation as targeted therapeutics for mono- and combination treatment HCC.

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Tumor microenvironment-dependent epigenetic imprinting in the vasculature predicts colon cancer outcome

Elisabeth Naschberger[1,2*], Maximilian Fuchs[3,4], Nicholas Dickel[3], Meik Kunz[3,4], Bernt Popp[5], Charles G. Anchang[6], Richard Demmler[1,2], Yanmin Lyu[1,2], Steffen Uebe[5], Arif B. Ekici[5], Carol I. Geppert[2,7], Arndt Hartmann[2,7], Christian Flierl[1], Katja Petter[1], Tobias Gass[1], Simon Völkl[8], Michael Scharl[9], Andreas Ramming[6], Claudia Günther[10], Susanne Merkel[2,11], Vera S. Schellerer[2,11], and Michael Stürzl[1,2]
[1]Division of Molecular and Experimental Surgery, Translational Research Center, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, 91054, Germany and [2]Comprehensive Cancer Center Erlangen-EMN (CCC ER-EMN), Erlangen, 91054; [3]Chair of Medical Informatics, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, 91054; [4]Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, 30625; [5]Institute of Human Genetics, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, 91054; [6]Department of Medicine 3, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, 91054; [7]Institute of Pathology, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, 91054; [8]Department of Medicine 5, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, 91054; [9]Department of Gastroenterology and Hepatology, University Hospital Zurich, University of Zurich, Zurich 8091, Switzerland; [10]Department of Medicine 1, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen 91054; [11]Department of Surgery, Universitätsklinikum Erlangen Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen 91054, Germany

Purpose: Antiangiogenic therapy is part of the guideline therapy of colorectal cancer (CRC). Surprisingly, the impact of the tumor microenvironment (TME) on tumor vessel endothelial cells (TECs) is largely unclear. The aim of this study was to investigate the presence of a TME-dependent transcriptional imprinting in TECs isolated from patients and to exploit it to retrieve signatures that characterize TECs in different intratumoral TMEs with impact on patient outcome.
Methods: ECs from tumor and normal colon tissues, PBMCs and tumor cells were isolated from CRC patients with different prognostic TMEs. Cells were analyzed by qPCR, immunocytochemistry and multi-omics (transcriptomics, EPICmethylation chips, exome sequencing). Integrative bioinformatics was used to identify TME-dependent imprinting genes predicting prognosis and scRNASeq to validate endothelial gene expression in CRC tissues.
Results: Ultrapure TECs were isolated from CRC with different prognostic TMEs (Th1 vs. non-Th1) and systematically compared by multi-omics. Transcriptional imprinting differentiating the respective TEC groups was identified. This *in vivo* transcriptional imprinting was preferentially regulated by epigenetic DNA methylation but not by genomic alterations and was different from an *in vitro* primed transcriptional memory to IFN-γ. Moreover, it was specific for TECs and not observed in CAFs. With integrative bioinformatics a TME-dependent imprinting signature was extracted and its expression in TECs in CRC tissues was confirmed by scRNASeq. Notably, the identified signature predicted the prognosis of CRC patients.
Conclusion: We identified a tumor vessel-derived TME-dependent transcriptional imprinting signature that was manifested by epigenetic mechanisms and allowed tumor vessel-based prediction of CRC patients prognosis.

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Aged garlic extract and S-allyl cysteine reduce the content of pro-inflammatory mRNAs in bronchial epithelial IB3-1 cells treated with SARS-CoV-2 Spike protein and BNT162b2 vaccine

Jessica Gasparello[1], Chiara Papi[1], Giulia Breveglieri[1], Matteo Zurlo[1], Alessia Finotti[1], Roberto Gambari[1] and Enzo Agostinelli[2,3]
[1]Department of Life Sciences and Biotechnology. Ferrara University; 44121 Ferrara, Italy
[2]Department of Sensory Organs, Sapienza University of Rome, Policlinico Umberto I, Viale del Policlinico 155, 00161 Rome, Italy
[3]International Polyamines Foundation-ONLUS, I-00159 Rome, Italy
E-mail: gam@unife.it

Garlic (*Allium sativum* L.) is a species of the onion family, *Alliaceae* widely used in biomedical applications. AGE (Aged garlic extract) is prepared by immersing fresh garlic in 15% aqueous ethanol solution over a prolonged period of time (1). The objective of this study was to determine the effects of AGE on proinflammatory genes, including those coding for proteins involved in the COVID-19 "Cytokine Storm". In the first experimental system we treated bronchial epithelial IB3-1 cells with SARS-CoV-2 Spike protein (S-protein) as previously described (2) in the absence or in the presence of AGE (0.1, 0.5 and 1 mg/ml). In the second experimental system we treated IB3-1 cells with the anti-SARS-CoV-2 BNT162b2 vaccine. After three days, cells were harvested and RT-qPCR and Bio-plex analysis performed to determine the expression of the IL-1β, IL-6 and IL-8. genes The results obtained demonstrated that AGE is a potent inhibitor of the S-protein-induced and BNT162b2-induced expression of the IL-1β, IL-6 and IL-8 genes. Bio-plex analysis was performed focusing on the proteins IL-6 and IL-8, which were the highly induced cytokines. The results demonstrate that AGE reduced their release, in agreement with the RT-qPCR data. In order to identify a possible bioactive compound present within AGE, the effects of one of the major constituents (S-allyl cysteine, SAC) were studied on the same experimental model systems. The results obtained suggest that SAC is one of the AGE constituents responsible for inhibiting S-protein-induced pro-inflammatory genes. These results support the concept that both AGE and SAC deserve further experimental efforts to verify their effects on pro-inflammatory genes in SARS-CoV-2 infected cells.

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Acknowledgments: The present study was supported by the MUR-FISR COVID-miRNAPNA Project (Grant no. FISR2020IP_04128); the study was also partially supported by Wakunaga Pharmaceutical Co., Ltd. (Japan).

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Aberrant DNA methylation potentiates oncogenes' expression and disease progression in ovarian cancer

Dimcho Bachvarov
Département de Médecine Moleculaire, Université Laval, CRCHU-Q, Centre de Recherche en Cancérologie Québec PQ, Canada
Email: dimtcho.batchvarov@crchudequebec.ulaval.ca

Epithelial ovarian cancer (EOC) accounts for 4% of all cancers in women and is the leading cause of death from gynecologic malignancies. The molecular basis of EOC initiation and progression is still poorly understood. Previously, we have applied an epigenomics approach to investigate the possible implication of aberrant DNA methylation in EOC etiology. We used methylated DNA immunoprecipitation in combination with CpG island tiling arrays to characterize at high resolution the DNA methylation changes that occur in the genome of serous EOC tumors during disease progression. We found widespread DNA hypermethylation that occurs even in less invasive/early stages of ovarian tumorigenesis. In contrast, significant DNA hypomethylation was observed only in high-grade (G3) serous tumors. This approach led to the identification of novel EOC oncogenes, potentially modulated by epigenetic mechanisms (hypomethylation) in advanced EOC, and displaying implication in different mechanisms of EOC dissemination, including alterations in gene expression control (RUNX1, RUNX2), abnormal metabolism (BCAT1), aberrant O-glycosylation (GALNT3) and importantly, epithelial to mesenchymal transition (EMT) regulation (Ly75, GRHL2, HIC-5). These genes could represent new therapeutic targets and/or novel biomarkers indicative for EOC progression. Moreover, our data are indicative for the implication of aberrant DNA methylation in EMT-mediated EOC progression.

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Absorption and effects of SAMN@TA@BSAO nanohybrid on Caco-2 cells

Federica Tonolo[1], Lavinia Rutigliano[1,2], Graziano Rilievo[1], Massimiliano Magro[1], Maria Luisa di Paolo[3], Maria Pia Rigobello[4], Enzo Agostinelli[5,6], Fabio Vianello[1]
[1]Dept. of Compared Biomedicine and Food Science, University of Padova, Padova, Italy; [2]Dept. Molecular medicine, Sapienza Univesity of Rome, Rome, Italy; [3]Dept. Molecular medicine, University of Padova, Padova, Italy; [4]Dept. of Biomedical Sciences, University of Padova, Padova, Italy; [5]Department of Sensory Organs, Sapienza University of Rome, Policlinico Umberto I, Italy; [6]International Polyamines Foundation 'ETS-ONLUS', Rome, Italy
E-mail: federica.tonolo@unipd.it

Nanotechnology has emerged as a promising field of science for its several applications from technological devices to nanomedicine. In this research, bovine spermine amine oxidase (BSAO) was immobilized onto a nanomaterial composed of magnetic iron oxide nanoparticles (SAMNs) and, tannic acid (TA, present on the surface). In particular, the effects and the absorption of this ternary hybrid (SAMS@TA@BSAO) in Caco-2 cells were evaluated. First, Caco-2 were treated with increasing concentrations of SAMN@TA@BSAO for 24 h and then MTT assay was performed. A linear decrease of cell viability until reaching 50 μg/ml was evident in treated cells. To understand if nanohybrids can exert their effects entering the cells, ICP-OES analysis was performed revealing that the nanoparticles were present in cells proportionally to the treatment time. Then, endogenous polyamine levels were measured in Caco-2 cell in order to understand if they can interact with the nanohybrids. As a result, a consistent amount of spermine was present in this cellular model. Then, the production of reactive oxygen species (ROS) in cells treated with 35 μg/ml SAMN@TA@BSAO was assessed. It was noteworthy that ROS production increased in cells treated with the nanohybrid respect to the untreated control. This condition induces oxidative stress resulting in the activation of Keap1/Nrf2 pathway, confirmed by the decrease of Nrf2 levels in the cytosol estimated through WB analysis. As evident, protein-nanoparticle conjugation could be a key for the modulation of biological functions.

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Investigation into the validity of the MYC internal ribosome entry site and its role in resistance to stress

Rosa Bordone[1*], Devon Michael Ivy[1*], Rodrigo D'Amico[1], Martina Barba[1], Fiorella Di Pastena[2], Bianca Cesaro[3], Alessandro Fatica[3], Laura Di Magno[1], Sonia Coni[1], Enzo Agostinelli[4,5], Gianluca Canettieri[1,4,6]
[1]Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena 291, 00161, Rome, Italy; [2]McMaster University, Faculty of Health Sciences, [3]Dep. Of Medicine, 1200 Main St W, Hamilton (ON), Canada; Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy. [4]Department of Sensory Organs, Sapienza University of Rome, Policlinico Umberto I, Viale del Policlinico 151, 00155, Rome, Italy; [5]International Polyamines Foundation 'ETS-ONLUS', Rome, Italy; [6]Istituto Pasteur, Fondazione Cenci-Bolognetti, Sapienza University of Rome, Viale Regina Elena 291, 00161, Rome, Italy

The tumoral microenvironment is often hostile for cancer cells, since numerous cellular stressors are frequently encountered. Examples of these stresses include intrinsic factors such as ROS overproduction and glucose deprivation, as well as extrinsic factors such as chemotherapy. In cells encountering these stresses, many different signalling pathways are affected; however, many effects congregate on inhibiting cap-dependent translation, which consequentially demands the cell to utilize secondary mechanisms for protein synthesis. The internal ribosome entry site (IRES), an RNA element that allows for the association of ribosomes to mRNA in absence of canonical 5' cap binding, is considered an important mechanism utilized by cancer cells in surviving these unfavorable conditions. MYC is upregulated in 70% of human cancers and a key driver in colorectal cancer (CRC), the third most deadly cancer worldwide. MYC was identified with an IRES in its 5' UTR nearly 30 years ago and has been suggested mechanistically to provide resistance of cancer cells to stress. However, conflicting reports about the existence of this IRES have been shown, particularly due to the use of dicistronic reporter techniques employed. Here, to address the function of this genomic region in CRC, we employed a two-pronged approach. First, we generated RNA reporter constructs *in vitro* to measure IRES activity in a controlled method that avoided the pitfalls of earlier reports. Secondly, we utilized the CRISPR-Cas9 system to excise study the MYC IRES directly and studied the response of cells and in their responses to a variety of cellular stress in cell culture and *in vivo*.

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Endogenous morphine in mollusk and human: Neuro-immune modulation

Wei Zhu
State University of New York, College at Old Westbury, SCI Research Institute, New York, USA

Molluscan nerve tissue contains relatively low but physiologically significant levels of morphine that can increase in response to trauma and starvation. When exposed to tyrosine, L-Dopa, and tyramine, ganglia exhibited a notable dose and concentration-dependent increase in dopamine and morphine. Of particular interest, a CPY2D6 enzyme closely resembling the human variant was identified in molluscan ganglia. Additionally, it was found that human white blood cells (WBC) can synthesize morphine from similar precursors via CPY2D6. Further experiments demonstrated that morphine released from WBC can initiate nitric oxide signaling via the mu-opiate receptor. These studies offer compelling evidence that the biosynthesis of morphine in animals closely resembles that in plants, and this biosynthetic pathway is prevalent in various tissues in both animals and humans. The research also explores the pathophysiological modulation of morphine within the neuro-immune system of both animals and humans.

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Investigating the potential of plant-derived antimalarial drugs, Artemisinin and Artesunate, in alleviating long COVID symptoms

Allen Qian[1,2] and Wei Zhu[2,3]
[1]Cornell University; [2]SCI Research Institute; [3]State University of New York, College at Old Westbury, USA

Three years after the initial outbreak, COVID-19 continues to pose a substantial societal challenge, notably due to the persistence of long-lasting aftereffects, commonly referred to as Long COVID or post-acute sequelae of SARS-CoV-2 infection (PASC). Long COVID encompasses the enduring symptoms or emergence of new ones following recovery from the acute phase of COVID-19. This phenomenon carries significant implications, including heightened healthcare utilization, strain on hospital capacities, workforce ramifications, and mental health concerns. Consequently, addressing this issue requires intensified research efforts. This study aims to explore whether plant-derived antimalarial compounds, Artemisinin and Artesunate, may offer beneficial effects against the viral infection and inflammation associated with COVID-19. Known for their anti-viral and anti-inflammatory properties, as well as economic viability, these compounds were investigated using human immune cells expressing ACE2 receptors as a cellular model. Multiple assays were employed to assess their impact on immune response, including ELISA assays to measure cytokine expression, cell adhesion assays with ImageJ analysis for immune cell adhesion, and MTT, Caspase, and LDH assays to gauge immune cell viability, apoptosis, and necrosis. Additionally, computer-based molecular docking was employed to elucidate potential molecular mechanisms. Our results demonstrated that both Artemisinin and Artesunate significantly reduced IL1a expression induced by recombinant SARS-CoV-2 spike protein (SPK) for all concentrations ($p<0.05$). Furthermore, these compounds targeted cell adhesion molecules, effectively inhibiting immune cell adhesion. Moreover, Artemisinin and Artesunate mitigated SPK-induced apoptosis and necrosis ($p<0.05$). Computational analysis revealed that these compounds targeted crucial molecules involved in inflammation, infection, and pathological damage caused by the SARS-CoV-2 virus. This suggests that Artemisinin and Artesunate possess potent antiviral and anti-inflammatory properties. Further research is warranted to delve into the specific molecular pathways through which Artemisinin exerts its effects, providing a more comprehensive understanding of its potential in mitigating Long COVID symptoms.

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Chlorpyrifos: Effects on cell cytotoxicity, viability, and apoptosis via mitochondrial complex I and caspase III

Scout Chen, Wei Zhu
SCI Research Institute, Jericho, New York, USA

Chlorpyrifos (CPF) was a widely used organophosphate insecticide that was banned in the United States in 2021 and in the European Union in 2020 due to concerns related to neurotoxicity, genotoxicity, and apoptosis. However, previous studies on this chemical primarily focused on *in vitro* models involving rat neuronal cells and did not clearly define molecular targets. This study combines *in silico* molecular docking and *in vitro* human cell assays (LDH, metabolic activity, caspase) to gain a better understanding of how CPF affects human cells. CPF increased cytotoxicity rates in a dose-dependent manner, ranging from 5.5% to 12.7% for U937 cells ($p<0.05$) and from 1.8% to 9.4% in HTB11 cells ($p<0.05$). Additionally, CPF exposure led to a statistically significant decrease in cell viability at concentrations of 1 µM (29.7%), 100 µM (21.2%), and 10 µM (19.8%). In HTB11 cells exposed to CPF, viability exhibited a dose-dependent curve, peaking at a CPF concentration of 10 µM (29.9%). Caspase apoptotic activity reached its peak at concentrations of 0.1 µM (28%) and 100 µM (328%), respectively. Meanwhile, caspase activity in HTB11 cells followed a dose-dependent curve, with the highest activity at a concentration of 100 µM ($p<0.05$). *In silico* screening shown the two molecular targets of CPF with the highest binding affinity were mitochondrial complex I and caspase-3. This research suggests that environmental pollution caused by CPF may impact human neuronal systems by inducing caspase overactivity, leading to apoptosis, and affecting metabolism through interactions with mitochondrial complex I, thereby inhibiting cellular respiration. This study underscores the importance of implementing protective measures commensurate with the level of CPF contamination.

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Aloin induces cytotoxicity and generates cell death in lymphoma cells through the TNF pathway

Edward Huang, Wei Zhu
SCI Research Institute, 420 Jericho TPKE, Jericho, NY 11753, USA

Aloin, the major anthraquinone in aloe exudates and gels, has been reported to exhibit antitumor effects in previous studies. To understand the ability and mechanisms of aloin in inducing apoptosis and cell deaths, we used U937 lymphoma cells, treated with or without aloin, to assess the cell survival, and tried to uncover the pathway of apoptosis in the U937 cells after treatment with aloin. U937 lymphoma cells were treated with aqueous aloin for 24 hours and 48 hours. The MTT assay was performed to evaluate the survival rate of the U937 cells. The level of necrosis of the cells was determined by the LDH assay. The level of cellular apoptosis was determined by the caspase assay. Furthermore, to investigate the pathway of apoptosis, the PyRx computer program was used to simulate the bonds between TNF-alpha, TNF-receptor, and aloin. Our results have shown that aloin diminished the survival rate of all samples of U937 cells by using the MTT assay. The LDH assay results showed that necrosis was present only after 48 hours; while the caspase assay results showed that after 24 hours, aloin only induced apoptosis at a specific dosage. These data suggested that aloin may function in a timely and dosage related manner. Moreover, molecular docking revealed that aloin replaced TNF-alpha's spot in apoptosis. RNAseq analysis indicated Aloin regulated a serious of gene that related with cancer proliferation and migration. These findings revealed that aloin generated cell death in U937 cells through the process of both apoptosis and necrosis. In addition, the statistical insignificance in the data showed that TNF-alpha pathway was involved in aloin induced apoptosis and necrosis.

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Discovering apoptotic drug targets for colorectal cancer through innovative soft voting machine learning approach

Yifu Zuo and Wei Zhu
SCI Research Institute, Jericho, New York, USA

As of 2023, colorectal cancer (CRC) is the second most common cause of cancer death in the United States. The recent development of certain drugs that directly target specific apoptotic proteins or other molecules involved in cancer cell growth and survival has offered promising avenues for CRC treatment. The effectiveness of targeted drugs can differ among patients with CRC due to the variation in the genetic makeup of tumor cells. Machine Learning can automate the process of identifying potential protein drug targets with high accuracy and efficiency by analyzing large sets of protein information and determining whether they have apoptotic properties. In this study, a novel soft voting machine learning classifier was created to find potential apoptotic drug targets for CRC, which combines the predictions of individual machine learning models, namely Logistic Regression, MLP, SVC, and Naive Bayes, into a single weighted majority vote system that produces combined predictions on the apoptotic properties of proteins. By combining a diverse range of models, the soft voting classifier is able to improve the performance and balance out the weaknesses of machine learning models. Overall, the voting model achieved an accuracy of 83.4% and an F1 score of 83%, demonstrating the effectiveness of soft voting in apoptotic protein drug target identification.

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Tumor-acquired somatic mutation disrupts the TMD-NBD communication and transport

Tomoka Gose[1], Ali Rasouli[2], Sepehr Dehghani-Ghahnaviyeh[2], Po-Chao Wen[2], Yao Wang[1], John Lynch[1], Yu Fukuda[1], Talha Shafi[3], Robert C. Ford[3], Emad Tajkhorshid[2] and John D. Schuetz[1]
[1]Department of Pharmacy and Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis; [2]Department of Biochemistry, and Center for Biophysics and Quantitative Biology, University of Illinois at Urbana-Champaign, Urbana, USA; [3]School of Biological Sciences, The University of Manchester, Manchester, UK
E-mail: john.schuetz@stjude.org

ATP-binding cassette sub-family G member 2, ABCG2, exports many structurally diverse and mechanistically unrelated chemotherapeutic drugs from cells using the energy of ATP hydrolysis, resulting in their reduced efficiency. ABCG2 is also a key drug transporter that can alter the pharmacokinetics of its substrate drugs. We recently demonstrated a basis for ABCG2 broad substrate selection (Gose et al *Nature Comm*, 2023). ABCG2 somatic mutations might affect therapeutic efficacy and survival and such mutations in the phylogenetically conserved amino acids of ABCG2 might provide unique insights into its molecular mechanisms of transport. Studying the ABCG2 genomic alterations in pediatric cancer using the ProteinPaint data portal (https:// proteinpaint.stjude.org) revealed a novel somatic mutation, Q393K, that is highly conserved across mammalian species in a sonic-hedgehog-driven medulloblastoma patient. This ABCG2 mutant retained appropriate protein expression and substrate recognition. However, it was incapable of transporting any of the tested substrates. A conformationally sensitive antibody revealed that this mutation had an impaired substrate-driven transition from the inward-facing state to the outward-facing state. Structural modeling and molecular dynamics simulations, based on ABCG2 cryo-EM structures, revealed that this 393K forms a strong salt bridge with the E446 residue. We propose that this salt bridge stabilizes the inward-facing conformation, resulting in an impaired inflexible transporter that cannot readily change conformation, thereby disrupting the communication between substrate binding and transport.

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Investigation of mechanisms of direct cytotoxicity of anti-GD2 ganglioside monoclonal antibodies using neuroblastoma cell cultures

Irena Horwacik and Hanna Rokita
Laboratory of Molecular Genetics and Virology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7 St., Kraków, Poland
E-mail: irena.horwacik@uj.edu.pl

Neuroblastoma (NB) is a childhood malignancy arising from differentiation failure of neural crest progenitor cells of the sympatho-adrenal linage. It is the most common extracranial tumor in childhood. Despite advances in diagnosis, risk and treatment stratification, further efforts must be made to improve survival rates of high risk (HR) NB patients. This involves development of new drug combinations, including immunotherapies. Gangliosides are glycosphingolipids composed of ceramide and the sialic acid-containing sugar part[1]. They accumulate in the outer cell membrane and can participate in cell signaling. GD2 is overexpressed in NB and hence serves as a marker used in diagnosis of the disease, and an important target in treatment of HR NB patients with clinically approved monoclonal antibodies (mAb), i.e., dinutuximab, dinutuximab beta, naxitamab[1]. We observed that in cell cultures of some NB cell lines addition of anti-GD2 mAb can cause direct cytotoxic effects leading to cell death[2,3]. Our current research focuses on elucidation of molecular mechanisms regulating cell fate that are induced after treatment with 14G2a mAb and dinutuximab beta *in vitro*, using 2D and 3D cell culture models. We aim to broaden knowledge on how GD2 can regulate survival, migration, and cell interactions, but also which molecules are involved in transmission of signals after anti-GD2 mAb-treatment. This can aid development of GD2-specific immunotherapies and new agent combinations to improve outcomes of HR NB patients.

This research was supported by a grant from National Science Center (grant no. 2018/29/B/NZ7/01564) and by funds form the Ministry of Education and Science in Poland.

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Effects of silencing of the *PHLDA1* gene on gene expression and metabolism of human neuroblastoma cells

Beata Bugara[1], Małgorzata Durbas[1], Maja Kudrycka[1,2], Agata Malinowska[3], Irena Horwacik[1], Hanna Rokita[1]
[1]Laboratory of Molecular Genetics and Virology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland; [2]Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland; 3 Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
E-mail: hanna.rokita@uj.edu.pl

Neuroblastoma (NB) is the most common extracranial pediatric solid tumor originating from the abnormal development of cells of the sympathoadrenal lineage of the neural crest. Targeting GD2 ganglioside (GD2), a glycolipid expressed on neuroblastoma cells, with GD2 ganglioside-recognizing antibodies, including their chimeric form ch14.18, constitute now an important way to treat drug resistant form of high-risk neuroblastoma. Previously, we have found, that *PHLDA1* (pleckstrin homology-like domain family A member 1) gene as the most upregulated gene in the IMR-32 human neuroblastoma cells treated with the mouse 14G2a monoclonal antibody. Now, mass spectrometry-based proteomic analyses were applied to better characterize a role of PHLDA1 protein in the response of neuroblastoma cells to treatment with chimeric ch14.18/CHO antibody. Mass spectrometry analysis of the proteins co-immunoprecipitated using anti-PHLDA1-specific antibody, selected a group of possible PHLDA1 binding partners. One of them are DCAF7 and AUTS2 proteins forming a complex constituting a key component of neuronal differentiation process *in vitro*. Moreover, global protein expression profile analysis in the IMR-32 cell line with *PHLDA1* silencing revealed the increase in biological functions of mitochondria, accompanied by differentiation-like phenotype of the cells. Importantly, our results indicate that PHLDA1 silencing might weaken the mesenchymal and enhance adrenergic state of neuroblastoma cells.

This research was funded by a grant from National Science Center (to Hanna Rokita, grant no. 2018/29/B/NZ7/01564)

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Smoldering inflammation sustains tissue structure changes and tumor microenvironment development

Luca Vannucci[1], Fabián Čaja[1], Dmitry Stakheev[1,8], Pavol Lukác[1,2], Lenka Rajsiglova[1,2], Paolo Tenti[1,2], David Vondrasek[3], Gianluca Mucciolo[4], Renata Štěpánková[5], Peter Makovický[6], Pavol Makovický[7], Tomas Hudcovic[5], Petr Šima[1], Daniel Smrz[1,8]
[1]Institute of Microbiology of the CAS, Prague; [2]Faculty of Science, Charles University, Prague; [3]Institute of Physiology of the CAS, Prague, Czech Republic; [4]University of Torino, Torino, Italy; [5]Institute of Microbiology of the CAS, Nový Hrádek, Czech Republic; [6]University of Ostrava, Ostrava, Czech Republic; [7]J. Selye University, Komárno, Slovakia
E-mail: vannucci@biomed.cas.cz

Collagen accumulation can affect the local immunity as a physical impediment and as a T-cell inhibitor through LAIR-1 binding. Collagen organization is modeled by the local immunity. In a rat model of chronic colitis (dextran sodium sulphate – DSS) and of carcinogenesis (azoxymethane) we have shown rising of pro-inflammatory activities and remodeling of the collagen scaffold in both models. The process is active even when the mucosa appears recovered from the acute induction. IL-6 is the main player. The lowering of the "inflammatory threshold" of the mucosa, i.e. reduction of regulatory factors (TGF-β) levels, provides maintenance of inflammation even under apparently normal levels of tissutal cytokines. This generates a smouldering inflammation with damage the tissue structure. IL-17 is also relevant for the profibrotic collagen organization as shown in mouse pancreatic cancer models. The deregulated inflammatory threshold can allow smouldering inflammation and supporting chronic inflammation and/or cancer niches establishment.

Acknowledgements: the work was supported by Institutional Grant RVO 61388971 (CZ), GAAV IAA500200917 (CZ), MEYS CR (Large RI Project LM2018129 Czech-BioImaging), ERDF (project No. CZ.02.1.01/0.0/0.0/18_046/0016045) and the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union –Next Generation EU, Generali Česka Pojistovna Foundation (CZ), UniCredit Bank (CZ), SIAD s.r.o. (ITA/CZ), ARPA Foundation (ITA).

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Iron oxide-based nanohybrids as universal carriers for cellular delivery

Graziano Rilievo[1], Federica Tonolo[1], Lavinia Rutigliano[2,3], Massimiliano Magro[1], Maria Luisa di Paolo[4], Manuela Cervelli[5], Andrea Ilari[6], Anastasia Liuzzi[6], Enzo Agostinelli[3,7], Fabio Vianello[1,7]
[1]Department of Comparative Biomedicine and Food Science, University of Padua, Legnaro; [2]Department of Molecular Medicine, Sapienza University of Rome, Rome; [3]Department of Sensory Organs, Sapienza University of Rome, Rome; [4]Department of Molecular Medicine, University of Padua, Padua; [5]Department of Sciences, Roma Tre University, Rome; [6]Institute of Molecular Biology and Pathology, Italian National Research Council IBPM-CNR, Rome; [7]International Polyamines Foundation 'ETS-ONLUS', Rome, Italy
E-mail: graziano.rilievo@phd.unipd.it

Different nanomaterials play an unpaired role as platforms to manufacture drug delivery systems, thanks to their ability to be easily internalized by cells, especially cancer cells. In this context, our group developed peculiar metal oxide nanoparticles, named *Surface Active Maghemite Nanoparticles* (SAMNs), with ideal features for applications in biomedicine, such as high colloidal stability, superparamagnetism, and strong affinity for bio-macromolecules. Here, we present three different SAMN@enzime nanohybrids integrating enzymatic catalysis and magnetic properties aimed at enzyme delivery into cells. Enzymes were selected to catalyse the generation of cytotoxic molecules once inside cells. (i) a SAMN based ternary complex comprising tannic acid (TA) functionalized with bovine serum amine oxidase (BSAO), called SAMN@TA@BSAO, which oxidized polyamines to aldehydes and H2O2; (ii) mouse spermine oxidase (SMOX) directly bound on the surface of bare SAMNs resulting in a SAMN@SMOX hybrid which catalysed the oxidation of spermine; and finally (iii) SAMN surface was loaded with E3-ligase (E3Lig), one of the actors of the ubiquitin-proteasome system, which induces protein degradation. Experimental results confirmed that the nano-bio hybrids retained the catalytic activity of the enzymes, suggesting their potential as therapeutic agents. These three SAMN@enzyme conjugates represent a clear example of the unparalleled contribution that nanotechnology, and in particular SAMNs, could bring in the field of molecular medicine.

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Cytotoxic effects of magnetic nanohybrids on colorectal adenocarcinoma cell models

Lavinia Rutigliano[1,2], Federica Tonolo[2], Graziano Rilievo[2], Massimiliano Magro[2], Maria Luisa di Paolo[3], MariaPia Rigobello[4], Gianluca Canettieri[1], Enzo Agostinelli[5,6], Fabio Vianello[2,6]
[1]Dept. Molecular medicine, Sapienza University of Rome, Laboratory Affiliated to Istituto Pasteur Italia, Fondazione Cenci Bolognetti, Sapienza University of Rome; [2]Dept. Comparative Biomedicine and Food Science, University of Padova; [3]Dept. Molecular Medicine, University of Padova; [4]Dept. Biomedical Sciences, University of Padova; [5]Dept. Sensory Organs, Sapienza University of Rome, Policlinico Umberto I; 6International Polyamines Foundation 'ETS-ONLUS', Rome, Italy
E-mail: lavinia.rutigliano@uniroma1.it

Polyamines are involved in several cellular functions, and they are substrates of amine oxidases, a class of enzymes present in numerous living systems. The dysregulation of polyamine metabolism is an event associated with various pathological conditions, including cancer. In particular, high concentrations of polyamines and their biosynthetic enzymes are strongly associated with rapidly growing tumors, including breast, colon, prostate, and gastric cancers. Co-administration of spermine and amine oxidase from bovine serum (BSAO) was previously reported to induce a significant cytotoxic effect in colorectal cancer cells, via the production of cytotoxic H_2O_2 and aldehydes. For therapeutic purposes, the enzyme was conjugated to magnetic iron oxide nanoparticles (SAMN@TA@BSAO) (1) to allow the delivery of BSAO inside cells, leading to endogenous polyamines oxidation. We used three models of colorectal adenocarcinoma LoVo WT, LoVo DX (a multidrug resistant cell line), and Caco2 cell lines. SAMN@TA@BSAO activity was evaluated before and after administration to cell lines. Results showed that SAMN@TA@BSAO nanohybrids were efficiently delivered in all cell models and they were able to induce a significant decrease of cell viability, compared to untreated cells. Indeed, nanoparticles without bound BSAO did not show any cytotoxic effect, demonstrating the biocompatibility of the delivery system.

(1) Rilievo, G. *et al*. Int J Mol Sci 23, 12172 (2022).

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CyTOF and microRNAs reveal possible biomarkers for early diagnosis and treatment of Heparin Induced Thrombocytopenia (HIT)

Evangelia Kourepini[1*], Giannoula Soufla[2*], Eugenia Iliopoulou[2], Stavros Georgantis[2], Theoni Kanellopoulou[2], Dimitrios Boumpas and Theodora Kostelidou [2]
[1]Laboratory of Cellular Immunology, Biomedical Research Foundation, Academy of Athens, Athens; [2]Laboratory of Hematology, Onassis Cardiac Surgery Center, Athens and [3]Laboratory of Immune Regulation and Tolerance, Autoimmunity and Inflammation, Biomedical Research Foundation, Academy of Athens, Athens, Greece
*Contributed equally
E-mail: soufla@ocsc.gr

Acute and life-threatening complications are common in 2-5% of heparin administered patients. HIT is well correlated with the danger of arterial or vein thrombosis. Cardiothoracic patients are of high danger for HIT development as they have been repeatedly treated with heparin due to previous angiography. HIT syndrome is due to IgG antibody (Ab) formation against heparin-PF4 complexes causing strong activation and cross-linking of platelets through Fc receptors (FcγRIIa). Only a fragment of these patients develops "functional" Abs that can activate platelets and lead to thrombosis. Platelet microRNAs have been shown to contribute to Ab-mediated activation, the mechanism of platelet recruitment and clot formation, as well as to control immune responses that lead to HIT. Our findings demonstrate significantly enhanced expression of miR148a in the plasma of patients with functionally active HIT Abs (HIT-pos), when compared with patients with non-active Abs, or patients without HIT-specific Abs which serve as negative controls. Moreover, platelets of HIT-pos patients had increased TULA-2 mRNA expression with a concomitant decrease in FcγRIIa mRNA expression, demonstrating that these genes interrelate and regulate each other. The HIT-pos group had also increased platelet expression of CD40L and plasma levels of pro-inflammatory cytokines TNF-α and IL-6. CyTOF analysis of all patient groups, employing 31 markers, demonstrated B-cells to be the major players in thrombosis induction. Our results indicate that the number of B-cells over-expressing CD40 as well as miR148a could be utilized as potential biomarkers for HIT prevention and treatment.

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Combined treatment with pre-miR-93 and products from *Allium sativum* reduces interleukin-8 mRNA content induced by anti-SARS-CoV-2 BNT162b2 vaccine in bronchial epithelial IB3-1 cells

Chiara Papi[1], Jessica Gasparello[1], Giulia Breveglieri[1], Matteo Zurlo[1], Alessia Finotti[1], Roberto Gambari[1] and Enzo Agostinelli[2,3]
[1]Department of Life Sciences and Biotechnology. Ferrara University; 44121 Ferrara, Italy
[2]Department of Sensory Organs, Sapienza University of Rome, Policlinico Umberto I, Viale del Policlinico 155, 00161 Rome, Italy
[3]International Polyamines Foundation-ONLUS, I-00159 Rome, Italy
E-mail: chiara.papi@unife.it

Garlic (*Allium sativum* L.) has been widely used as a food and as a folk medicine. Among a large diversity of commercially available garlic supplements, AGE (Aged garlic extract) has been carefully studied (1). We have previously demonstrated that the agomiR pre-miR-93-5p is a potent inhibitor of Interleukin-8 (IL-8, a key components of the COVID-19 "Cytokine Storm") in bronchial epithelial cells induced to the activation of pro-inflammatory genes by exposure to SARS-CoV-2 Spike (S-protein) (2). The objective of this study was to determine the anti-inflammatory effects of combined treatments based on the co-administration of pre-miR-93-5p and AGE. To this aim we treated bronchial epithelial IB3-1 cells with the COVID-19 BNT162b2 vaccine in the absence or in the presence of pre-miR-93-5p (200 nM), AGE (0.5 mg/ml) or a combination of pre-miR-93-5p and AGE. We found that both pre-miR-93-5p and AGE retain inhibitory effects on proinflammatory genes induced by the BNT162b2 vaccine. In addition, RT-qPCR analysis demonstrated that the highest inhibitory effects were obtained with the combined treatment. These results suggest that this combined strategy deserves further experimental efforts to verify its effects on other pro-inflammatory genes, not only in cells treated with the BNT162b2 vaccine, but also in SARS-CoV-2 infected cells. Furthermore, compounds isolated from AGE (such as S-allyl cysteine and S-1-propenylcysteine) are expected to cooperate with the agomiR-93-5p in inhibiting expression of genes coding for proteins involved in the COVID-19 "Cytokine Storm".

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Acknowledgments: The present study was supported by the MUR-FISR COVID-miRNAPNA Project (Grant no. FISR2020IP_04128); the study was also partially supported by Wakunaga Pharmaceutical Co., Ltd. (Japan).

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Oncogenic potential of AAA+ ATPase proteins in cancer

Aditi Nayak
Department of Life Science, Guru Nanak Institute of Pharmaceutical Science and Technology, 157/F, Nilgunj Rd, Sahid Colony, Panihati, Kolkata, West Bengal-700114, India
E-mail: aditi.nayak@gnipst.ac.in

AAA+ ATPases (**A**TPase **A**ssociated with various cellular **A**ctivities) are a large family of mechanoenzymes. They exert their activity through energy-dependent processes. They are involved in vital cellular processes like cell cycle control, DNA replication, cytoskeleton dynamics, membrane fusion, signal transduction and the regulation of gene expression. Therefore, they play important roles in a wide range of diseases including cancer. Our group is working on these AAA+ ATPases and has revealed the role of ATAD2 (ATPase family AAA domain-containing 2) and NVL2 (Nuclear VCP-like protein) like ATPases in different cancers. By systematic analyses with relevant experimental studies, here we evaluate for the first time the prospect of *ATAD2* and *NVL2* as a diagnostic and prognostic biomarker for cancers. Our cross-cancer analyses reveal that these proteins are overexpressed in many cancers and is related to poor survival of the cancer patients. Most interestingly, *ATAD2* appears as the cancer driver for many cancers and the potential mutation sites of ATAD2 are predicted. These studies across the cancer types specify ATAD2 as an interesting target particularly for stomach cancer. Therefore, we further focus our study on stomach cancer and indeed find that *ATAD2* is overexpressed in all stages and grades of stomach adenocarcinoma. Since, hypoxia is one of the most crucial factors in Stomach Cancer, we elucidated the function of ATAD2 in hypoxic Gastric epithelial cells. We have revealed ATAD2 as hypoxia responsive HIF1α regulated protein. Therefore, the analysis reported here establishes *ATAD2* as a very promising biomarker and therapeutic target for stomach cancer.

Keywords
AAA+ ATPase, ATAD2, NVL2, Biomarker, Prognostic Marker.

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Involvement of autophagy as detoxifying mechanism in erythroid cells of β-Thalassemia patients accumulating free α-globin

Matteo Zurlo[1], Cristina Zuccato[1,2], Lucia Carmela Cosenza[1], Chiara Papi[1], Giulia Breveglieri[1], Alessia Finotti[1,2], Roberto Gambari[1,2]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, and [2]Center 'Chiara Gemmo and Elio Zago' for the Research on Thalassemia, University of Ferrara, Italy
E-mail: matteo.zurlo@unife.it

β-thalassemias are a group of inherited blood disorders caused by a variety of mutations of the gene encoding for β-globin. The low or absent production of β-globin leads to an excess in the content of free α-globin chains, that precipitates and forms toxic insoluble aggregates in erythroid precursor cells (ErPCs), causing their premature death (hemolysis) and subsequent inefficient erythropoiesis. In this respect, the mTOR inhibitor Sirolimus (Rapamycin) is a repositioned drug of great interest. It is well known that mTOR inhibitors can induce autophagy, but whether this process can be exploited to detoxify ErPCs from α-globin accumulation remains unclear. To verify autophagy involvement in α-globin accumulation, we produced and characterized K562 clones able to express α-globin protein at high levels. In addition, we tested the potential of Sirolimus to induce clearance of α-globin chains via autophagy triggering in erythroid precursor cells isolated from β-thalassemia patients. In this study, we demonstrated that the autophagy process is strictly related to α-globin accumulation in K562 cellular clones forced to hyper-express toxic α-globin. Moreover, Sirolimus showed efficacy in reducing α-globin excess through activation of the autophagy process. This was found both ex vivo, on ErPCs cells isolated from βThalassemia patients and treated with low dosages of Sirolimus, and in vivo, in β-Thalassemia patients recruited for the SIRTHALACLIN clinical trial (NCT03877809) and treated with 0.5-2 mg/day Sirolimus.

Supported by Welcome Trust (United Kingdom, Innovator Award 208872/Z/17/Z), by the UE FP7 THALAMOSS Project (grant n.306201-FP7-Health-2012-INNOVATION-1) and by AIFA (Agenzia Italiana del Farmaco, Italy, AIFA-2016-02364887).

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Glucocorticoid effects on normal brain tissue

Stanislav D. Aladev[1], Dmitry K. Sokolov[1], Galina M. Kazanskaya[1], Akexander M. Volkov[2], Maxim O. Politko[1], Anastasia V. Strokotova[1], Svetlana V. Aidagulova[1,3], Elvira V. Grigorieva[1]
[1]Institute of Molecular Biology and Biophysics FRC FTM; [2]Meshalkin National Medical Research Center; [3]Novosibirsk State Medical University, Novosibirsk, Russia
E-mail: elv_grig@yahoo.com

Glucocorticoids are used for the treatment of numerous pathological conditions including neurodegenerative and inflammatory diseases, cancers and COVID-19. However their long-term administration leads to significant side-effects, which molecular mechanisms are not enough studied. Here, we investigated long-term effects of dexamethasone (DXM) towards a composition and structure of brain extracellular matrix, which mainly compose of such glycosylated macromolecules as proteoglycans (PGs) and glycosaminoglycans (GAGs). Single and multiple administration of various DXM doses to 2-month-old C57BL/6 mice was done, with subsequent analysis of the expression of glucocorticoid receptor (GR), PGs, heparan sulfate (HS) biosynthetic enzymes (RT-PCR) as well as GAG content (dot-blot, Alcian Blue staining) in the brain tissue at the 3-90 days time period. Both a single and multiple DXM administration led to fast activation of GR, PGs, HS enzymes expression (+1.5-3-fold) in the mouse brain, with return to control values by 7-10 (single) or 30-60 (multiple) days after DXM administration. GR expression demonstrated high correlation with that of PGs and HS biosynthesis-involved genes (P=0.81-0.97, for 20 out of 28 genes), which mitigated under the DXM pressure. At the same time, DXM effect towards GAGs content was more delayed and stable, with a dose-dependent increase of low-sulfated GAGs and high-sulfated GAGs in brain tissue (starting from days 1 and 3-7, respectively), with a complete return to baseline values only by 60-90 days after DXM administration. In summary, DXM leads to the fast short-termed activation of the expression of the studied genes but prolonged increase of the GAGs content in the mouse brain, which can lead to the changes in the composition and structure of the brain tissue, as well as its functional characteristics.

This study was funded by the Russian Science Foundation (grant RSF № 21-15-00285).

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Molecular determinants of drug resistance in colorectal cancer organoids and novel integrated treatment approaches

Luca Falzone, Graziana Spoto, Daria Ricci, Saverio Candido, Massimo Libra
Experimental Oncology Laboratory, Department of Biomedical and Biotechnological Sciences, University of Catania, 95123 Catania, Italy
Email: luca.falzone@unict.it

Colorectal cancer (CRC) is one of the most lethal tumors worldwide, mainly due to late diagnosis and treatment failure. Standard chemotherapy with the combination of 5-fluorouracil (5-FU), oxaliplatin (OX), or irinotecan (IRN) represents the first-line treatment for CRC patients, however, the majority of patients develop drug resistance that renders the treatments ineffective with mechanisms that have not been elucidated yet. On this basis, the main objective of the study was to generate advanced drug-resistant CRC models using patient-derived tumor organoids (PDTOs) to fully characterize the molecular landscape of CRC resistance and propose new integrated approaches to overcome resistance mechanisms. To this end, three different drug-resistant CRC PDTOs were generated by treating sensitive organoids with increasing sublethal doses of 5-FU, OX and IRN for at least 90 days. After treatments, resistant PDTOs showed an increment of the IC50 values ranging from 2.92- to 14.70 depending on the drug used (5-FU: IC50sens=11.24 μM vs IC50res=165.3 μM; OX: IC50sens=43.06 μM vs IC50res=225.5 μM; IRN: IC50sens=4.59 μM vs IC50res=13.44 μM). In addition, DNA and RNA were obtained from both sensitive and resistant PDTOs to perform whole exome sequencing and transcriptomic analyses and determine molecular alterations associated with drug resistance. These analyses revealed genomic alterations, gene fusions, and altered gene expression affecting key cellular and molecular pathways involved in drug resistance, allowing the identification of molecular signatures responsible for the development of CRC 5-FU, OX- and IRN resistance. Finally, resistant PDTOs were treated with peptides derived from a probiotic strain, *Lactobacillus rhamnosus* GG, alone or in combination with standard chemotherapy. These experiments demonstrated how the administration of probiotics can reverse drug resistance in CRC and improve the efficacy of currently used pharmacological treatments. Overall, the results obtained here pave the way for further functional in vitro and in vivo studies aimed at validating the diagnostic or prognostic potential of the observed alterations and proposing new therapeutic targets for the development of effective tailored treatments in refractory patients.

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Novel insights into the epigenetic regulation of SLC22A17 in cutaneous melanoma. Validation of the cg17199325 methylation hotspot as potential diagnostic biomarker

Alessandro Lavoro[1], Luca Falzone[1], Giuseppe Gattuso[1], Giuseppe N. Conti[1], Massimo Libra[1,2], Saverio Candido[1,2]
[1]Department of Biomedical and Biotechnological Sciences; [2]Research Center for Prevention, Diagnosis and Treatment of Cancer, University of Catania, Catania, Italy
E-mail: alessandrolavoro@ymail.com

DNA methylation (methDNA) is the most characterized epigenetic mechanism. As widely reported, promoter hypomethylation is strictly associated to oncogene activation. Conversely, the role of intragenic methDNA has not been completely elucidated yet. In this field, genes involved in iron trafficking within the tumor microenvironment, including Gelatinase-Associated Lipocalin (NGAL) and its receptor Solute Carrier Family 22 Member 17 (SLC22A17), are finely modulated by methDNA. Starting from these observations, this study was aimed to investigate the role of methDNA in the regulation of SLC22A17 in cutaneous melanoma (CM). To this purpose, TCGA and GEO datasets were explored to evaluate epigenetic phenomena affecting SLC22A17. Validation analyses were performed analyzing the cg17199325 methylation hotspot of SLC22A17 in melanoma FFPE samples as putative diagnostic biomarker. Moreover, CM cells were treated with 5-azacytidine (5-aza) to assess the correlation between methDNA and SLC22A17 expression, whereas SLC22A17-A375 transfected cells were treated with ferroptosis activators to evaluate the SLC22A17 involvement. Computational analyses showed a significant downregulation of SLC22A17 in CM, whose expression levels were positively correlated with intragenic methDNA. Of note, the cg17199325 hotspot showed higher methDNA levels in FFPE melanoma samples compared to benign nevi. The correlation between methDNA and SLC22A17 expression was confirmed in 5-aza treated and untreated cells. Interestingly, A375 overexpressing SLC22A17 isoform 3 acquired resistance to iron overload and RSL-3. However, baseline sensitivity was restored by the concomitant overexpression of NGAL. Overall, the cg17199325 hotspot could represent a promising diagnostic biomarker for CM, highlighting the regulatory role of methDNA on SLC22A17 expression. In addition, the involvement of SLC22A17 isoform 3 in ferroptosis resistance paves the way for the identification of novel therapeutic strategies in CM treatment.

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Real-time observation of capsaicin-induced intramolecular domain dynamics of TRPV1 using the diffracted X-ray tracking method

Kazuhiro Mio[1], Tatsunari Ohkubo[1], Daisuke Sasaki[2], Tatsuya Arai[2], Mayui Sugiura[1], Shoko Fujimura[1], Yuji C. Sasaki[1,2]
[1]OPERANDO-OIL, National Institute of Advanced Industrial Science and Technology; [2]Graduate School of Frontier Sciences, The University of Tokyo, Japan
E-mail: kazu.mio@aist.go.jp

The transient receptor potential vanilloid type 1 (TRPV1) is a multimodal receptor that responds to various stimuli, including capsaicin, protons, and heat. Recent advancements in cryo-electron microscopy have revealed the structures of TRPV1. However, due to the large size of TRPV1 and its structural complexity, the detailed process of channel gating has not been well documented. In this study, we applied the diffracted X-ray tracking (DXT) technique to analyze the intramolecular dynamics of TRPV1. DXT is an X-ray-based real-time measurement technique with the highest spacio-temporal resolution among the currently available measurement technologies. DXT captures intramolecular motion through the analysis of trajectories of Laue spots generated from attached gold nanocrystals. The diffraction data were divided into two axes: twisting (χ) and tilting (θ), and they were analyzed separately. We successfully elucidated capsaicin-induced twisting motion at the extracellular domains, as well as the high-speed fluctuations at the C-terminal cytoplasmic domains. We also developed a lifetime-filtering technique that allowed us to segregate the different speed components from the mixed motion groups. Furthermore, the capsaicin-induced rotational bias was reversed between the wild-type and the capsaicin-insensitive Y511A mutant. This marks the first instance of real-time observation of the internal domain dynamics of TRPV1.

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