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International Society for Nitric Oxide and Cancer ISNOC

SOCIETÀ ITALIANA DI FARMACOLOGIA

IBiS INSTITUTODEBIOMEDICINADESEVILLA

SECOND INTERNATIONAL CONFERENCE

Therapeutic Applications of Nitric Oxide in Cancer and Inflammatory-related Diseases

(on-site and virtual)

Institute of Biomedicine of Seville

March 3-5, 2022, Seville, Spain

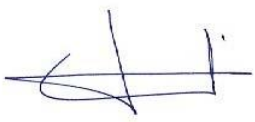
Dear Colleagues,
We are delighted you attend the Second International Conference “Therapeutic Applications of Nitric Oxide in Cancer and Inflammatory-related Diseases” (on-site and virtual) in Seville (Spain) from the March 3 to 5, 2022. The conference is held in the Institute of Biomedicine of Sevilla (IBiS) that includes researchers from different biomedical disciplines into an active and innovative research atmosphere.

The First International Conference on “Therapeutic Application of Nitric Oxide in Cancer and Inflammatory Disorders” was organized by Dr. Lucia Morbidelli and Dr. Benjamin Bonavida in Siena (Italy) on October 4-5, 2018. Since then, the field has significantly progressed and we, Dr. Lucia Morbidelli, Dr. Benjamin Bonavida and myself are co-organizing this Second International Conference on “Therapeutic Applications of Nitric Oxide in Cancer and Inflammatory-related Diseases”. The main focus of the conference is an update with presentations and discussions regarding the impact of nitric oxide-based drugs or nitric oxide-generating systems, under basic laboratory investigations or in clinical trials, with the overall objective to treat particularly cancer and inflammatory human diseases.

The present workshop is divided into six sessions specifically focused on the “Development of nitric oxide donors in targeting disease management”, “Nitric oxide disturbances in metabolic and cardiovascular disorders”, “Nitric oxide in hypertensive-related diseases”, “Nitric oxide and neurodegeneration” and “Acute and chronic inflammatory-related diseases”. Each session includes conferences presented by well-known researchers in the field, as well as young investigators presenting their data. In addition, two keynote lectures entitled “Hemeproteins, nitric oxide, and their relationships in cancer” by Dr. Dennis J Stuehr and “Nitric oxide-based therapeutic intervention in cancer” by Dr. David A Wink are included in the program. All registered researchers will have the opportunity to submit original research manuscripts, methods or graphical issues or full review articles to be published in a special book edited by Elsevier.

We hope that the workshop will allow scientific exchanges among researchers from different fields with an opportunity for valuable scientific discussions, initiating new research links and friendships as well as enjoying the culture of Sevilla, Andalucía and Spain.
I gratefully acknowledge the financial support received from the International Society of Nitric Oxide and Cancer (ISNOC), Institute of Biomedicine of Seville (IBiS), Elsevier/AP Publishing Company, University of Seville and the Società Italiana di Farmacologia (SIF). I would also to thank the members of my research group Elena Navarro, Patricia de la Cruz, Carlotta Pranzo and Thaissa Marins who have greatly help me during the meeting, as well as the secretariat of IBiS and Viajes Atlanta for their effectiveness.

Sincerely yours,



Jordi Muntané, Ph.D.
Professor of Physiology

Benjamin Bonavida, Ph.D.
Research Professor, UCLA

Lucia Morbidelli, Ph.D.
Professor of Pharmacology

Organizing Committee:

Jordi Muntané, PhD
Associate Professor
Department of Medical Physiology and Biophysics
University of Seville

Lucia Morbidelli, Ph.D
Professor of Pharmacology
Department of Life Sciences
University of Siena

Benjamin Bonavida, Ph.D
Professor
Department of Microbiology, Immunology,
and Molecular Genetics
University of California

Meeting link: https://isnoc.org/ii-therapeutic-application-of-no-in-cancer-and-inflammatory-diseases/

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PROGRAM

Day 1: March 3, 2022

16:00-16:15

Welcome and opening the meeting from the authorities

Rafael Fernández Chacón (Scientific Director of the Institute of Biomedicine of Seville) (ON-SITE)

Benjamin Bonavida (International Society of Nitric Oxide and Cancer, University of California) (ZOOM)

Jordi Muntané (Local Organizing Committee, Institute of Biomedicine of Seville) (ON-SITE)

OPENING SESSION I

KEYNOTE LECTURE 1

Moderator: Dr. Ben Bonavida (ZOOM)
(University of California, USA)

16:15-17:00 (ON-SITE)

Dennis J Stuehr (Department of Inflammation and Immunity, Lerner Research Institute, The Cleveland Clinic, Cleveland, USA)

Hemeproteins, NO, and their Relationships in Cancer

17:00-17:30 ***Coffee break***

SESSION 2

DEVELOPMENT OF NITRIC OXIDE DONORS IN TARGETING DISEASE MANAGEMENT

Moderator: Dr. Lucia Morbidelli (ON-SITE)
(University of Siena, Italy)

17:30-18:00 (ZOOM)

Sruti Shiva (University of Pittsburgh, Pittsburgh, USA)

Regulation of mitochondria and metabolism by NO and myoglobin in cancer and inflammation

18:00-18:30 (ZOOM)

Alia Shatanawi (School of Medicine, The University of Jordan, Amman, Jordan)

The role of the arginase-Nitric Oxide pathway in vascular dysfunction and inflammation

18:30-19:00 (ON-SITE)

Jean-Luc Balligand (Institut de Recherche Experimentale et Clinique (IREC) and Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium)

Nitric oxide signaling in cardiovascular health and disease

19:00-19:30
Young investigator session (10+5 min)

The metal-nonoate Zn(PipNONO)Cl exhibit antitumor activity through inhibition of epithelial and endothelial mesenchymal transitions (EMT and EndMT) (ZOOM)

Valerio Ciccone[1,2], Carlotta Pranzo[1], Arianna Filippelli[1], Enrico Monzani[3], Lucia Morbidelli[1]
[1]Department of Life Sciences, University of Siena, Siena, Italy; [2]Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy; and [3]Department of Chemistry, University of Pavia, Pavia, Italy

Interactions between Nitric Oxide and Hemin and their Implications in the Nitration of Proteins (ZOOM)

Amir Alsharabasy[1], Sharon Glynn[1,2], Pau Farràs[1,3], Abhay Pandit[1]
[1]CÚRAM, SFI Research Centre for Medical Devices; [2]Discipline of Pathology, Lambe Institute for Translational Research, School of Medicine, [3]School of Biological and Chemical Sciences, Ryan Institute, National University of Ireland, Galway

19:30-23:00 ***Dinner***

Day 2: March 4, 2022

SESSION 3
NITRIC OXIDE DISTURBANCES IN METABOLIC AND CARDIOVASCULAR DISORDERS
Moderator: Dr. Sharon Glynn (ON-SITE)
(National University of Ireland, Ireland)

8:00-8:30 (ZOOM)
Cyrille Boyer (Australian Centre for NanoMedicine or CAN, and Centre for Advanced Macromolecular Design or CAMD, School of Chemical Engineering, University of New South Wales, Sydney, Australia)
Engineering Polymeric NanoParticles for Advanced Applications

8:30-9:00 (ZOOM)
Chang-Ming Dong (School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai, China)
Photoresponsive polypeptide nanomedicines: from rational design to efficient NO gas delivery and cancer therapy

9:00-9:30 (ZOOM)

Arduino A. Mangoni (Department of Clinical Pharmacology, Flinders Medical Centre and Flinders University, Bedford Park, Australia)

Targeting dimethylarginine dimethylaminohydrolase to suppress vasculogenic mimicry in cancer: current evidence and future directions

9:30-10:00

Young investigator session (10+5 min)

Nitric oxide as an activator of HER2 in breast cancer (ZOOM)

Ciara O'Neill[1], Eoin Dervan[1], Jake McAuliffe[1], Suguna Sundararaman[1], Sharon Glynn[1]
[1]Discipline of Pathology, Lambe Institute for Translational Research, National University of Ireland Galway

Oxidative stress-induced endothelial dysfunction and decreased vascular nitric oxide in COVID-19 patients (ZOOM)

Virginie Montiel[1,2], Irina Lobysheva[2], Ludovic Gérard[1,3], Marjorie Vermeersch[4], David Perez-Morga[4], Thomas Castelein[1], Jean-Baptiste Mesland[1], Philippe Hantson[1], Christine Collienne[1], Damien Gruson[5], Marie-Astrid van Dievoet[5], Alexandre Persu[6,7], Christophe Beauloye[6,7], Mélanie Dechamps[1,7], Leïla Belkhir[8], Annie Robert[9], Marc Derive[10], Pierre-François Laterre[1], A.H.J Danser[11], Xavier Wittebole[1], Jean-Luc Balligand[2]
[1]Intensive Care Unit, Cliniques Universitaires Saint-Luc, Brussels, Belgium; [2]Institute of Experimental and Clinical Research, Pole of Pharmacology and Therapeutics, Université catholique de Louvain, Brussels, Belgium; [3]Institute of Experimental and Clinical Research, Pole of Pneumology, ENT and Dermatology, Université catholique de Louvain, Brussels, Belgium; [4]Center for Microscopy and Molecular Imaging, Université Libre de Bruxelles, Gosselies, Belgium; [5]Department of Laboratory Medicine, Cliniques Universitaires Saint-Luc, Brussels, Belgium; [6]Institute of Experimental and Clinical Research, Pole of Cardiovascular Research, Université catholique de Louvain, Brussels, Belgium; [7]Department of Cardiology, Cliniques Universitaires Saint-Luc and Université catholique de Louvain, Brussels, Belgium; [8]Department of Internal Medicine, Cliniques Universitaires Saint-Luc and Université catholique de Louvain, Brussels, Belgium; [9]Institute of Experimental and Clinical Research, Pole of Epidemiology and Biostatistics (EPID), Université catholique de Louvain, Brussels, Belgium; [10]Inotrem SA, Vandoeuvre-les-Nancy France, France; [11]Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands

10:00-10:30 ***Coffee break***

SESSION 4
NITRIC OXIDE IN HYPERTENSIVE-RELATED DISEASES
Moderator: Dr. Stéphanie Plenchette (ON-SITE)
(EPHE PSL-University of Burgundy, France)

10:30-11:00 (ZOOM)
Claudiu T. Supuran (Department of Neuroscience, Psychology, Drug Area and Child Health University of Florence, Florence, Italy)
Advances in the discovery of novel agents for the treatment of glaucoma

11:00-11:30 (ZOOM)
Alma Martelli (Department of Pharmacy, University of Pisa, Pisa, Italy)
The value of sulfur nutraceuticals for the cardiovascular inflamm-ageing: possible role of hydrogen sulfide

11:30-12:00 (ON-SITE)
María M. Miranda (Department of Biomedical Sciences, University CEU Cardenal Herrera, Alfara del Patriarca, Valencia, Spain)
Nitric oxide roles in retinal pathologies

12:30-13:00
Young investigator session (10+5 min)

Excessive *S*-nitrosylation induced by GSNOR deficiency increases malignancy in rhabdomyosarcoma (ZOOM)
Costanza Montagna[1], Chiara Pecorari[2], Paola Giglio[1], Valeria Fiorentini[1], Salvatore Rizza[2], Giuseppe Filomeni[1,2,3]
[1]Department of Biology, University of Rome Tor Vergata, Rome, Italy; [2]Redox Biology Group, Danish Cancer Society Research Center, Copenhagen, Denmark. [3]Center for Healthy Aging, University of Copenhagen, Denmark

Unraveling the role of AKR1A1 in renal and hepatocellular carcinoma progression (ON-SITE)
Chiara Pecorari[1], Giuseppe Filomeni[1,2]
[1]Redox Biology Group, Danish Cancer Society Research Center, Copenhagen, Denmark;
[2]Department of Biology, University of Rome Tor Vergata, Rome, Italy

13:00-15:00 ***Lunch***

SESSION 5
NITRIC OXIDE AND NEURODEGENERATION
Moderator: Dr. Giuseppe Filomeni (ON-SITE)
(Danish Cancer Society Research Center, Denmark)

15:00-15:30 (ZOOM)
João Laranjinha (Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal)

Modulation of neurovascular coupling in the brain by nitric oxide and cognitive enhancement

15:30-16:00 (ON-SITE)
Michael T Heneka (Luxembourg Centre for Systems Biomedicine-LCSB, University of Luxembourg, Luxembourg)
Inflammation in neurodegenerative disease: Role of iNOS and nitric oxide

16:00-16:30 (ON-SITE)
Joern R Steinert (Division of Physiology, Pharmacology and Neuroscience. Faculty of Medicine and Health Sciences, University of Nottingham, UK)
NO-mediated neuroinflammatory pathways as treatment targets in neurodegeneration

16:30-17:00 ***Coffee break***

SESSION 6
ACUTE AND CHRONIC INFLAMMATORY-RELATED DISEASES
Moderator: Dr. Khosrow Kashfi (ON-SITE)
(The City College of New York, USA)

17:00-17:30 (ZOOM)
Lisa A Ridnour (National Cancer Institute, Frederick, USA)
NOS and COX Inhibition Augments Immune Polarization and Improves Survival in Radiotherapy: Are NSAIDs and NOS inhibitors a New Approach to Immunotherapy?

17:30-18:00 (ZOOM)
Amedea B Seabra (Nanomedicine Research Unit, Federal University of ABC, Santo André, Brazil)
Nanomedicine allied to nitric oxide donors in cancer: Where are we and where can we go?

18:00-18:30 (ZOOM)
Daniel W McVicar (National Cancer Institute, Frederick, USA)
Nitric Oxide in Macrophage Immunometabolism: Evidence for HNO-mediated inhibition of PHD during activation

SESSION 7
KEYNOTE LECTURE 2
Moderator: Dr. Jordi Muntané (ON-SITE)
(Institute of Biomedicine of Seville, Spain)

18:30-19:15 (ZOOM)
David A Wink (National Cancer Institute, Frederick, USA)
NO-based therapeutic intervention in cancer

19:15-23:00 ***Dinner***

Day 3: March 5, 2022

SESSION 8
PROTECTIVE EFFECTS OF NUTRACEUTICALS: FOCUS ON NITRIC OXIDE AND OTHER GASOTRANSMITTERS
Moderator: Dr. Valentina Rapozzi (ON-SITE)
(University of Udine, Italy)

8:00-8:30 (ON-SITE)
Alex Dyson (Institute of Pharmaceutical Science, and Centre for Pharmaceutical Medicine Research King's College London, London, UK)
Selenium and hydrogen selenide: essential micronutrient and the fourth gasotransmitter?

8:30-9:00 (ON-SITE)
José M Palma (Department of Biochemistry, Molecular and Cell Biology of Plants, Estación Experimental del Zaidín, CSIC, Granada, Spain)
Does nitric oxide potentiate the anti-tumoral activity of pepper fruit extracts?

9:00-9:30 (ZOOM)
Chiara Riganti (Department of Oncology, University of Torino, Torino, Italy)
Nitric oxide-releasing gemcitabine: a new weapon against pancreatic cancer?

9:30-10:00 ***Coffee break***

10:00-11:00
Young investigator session (10+5 min)

Unraveling the role and regulation of S-nitrosoglutathione reductase in breast cancer progression (ON-SITE)
Valeria Fiorentini[1], Salvatore Rizza[1,2], Fiorella Faienza[1], Paola Giglio[1], Gennaro Pepe[1], Giuseppe Filomeni[1,2]
[1]Department of Biology, University of Rome Tor Vergata, Rome, Italy; [2]Redox Biology Group, Danish Cancer Research Center, Copenhagen, Denmark

TRAP1 regulation by NO and ROS highlight its potential role as a mitochondrial redox Sensor (ZOOM)

Fiorella Faienza[1], Salvatore Rizza[2], Chiara Pecorari[2], Valeria Fiorentini[1], Paola Giglio[1], Claudio Laquatra[3], Carlos Sanchez-Martin[3], Giovanni Chiappetta[4], Francesca Pacello[1], Andrea Battistoni[1], Elena Papaleo[5], Andrea Rasola[3], Giuseppe Filomeni[1,2,6]

[1]Department of Biology, University of Rome Tor Vergata, Rome, Italy. [2]Redox Biology Group, Danish Cancer Society Research Center, Copenhagen, Denmark. [3]Department of Biomedical Sciences, University of Padova, Padova, Italy. [4]Laboratory of Proteomics and Biological Mass Spectrometry, USR, CNRS - ESPCI Paris, Paris, France. [5]Computational Biology Laboratory, Danish Cancer Society Research Center, Copenhagen, Denmark. [6]Center for Healthy Aging, University of Copenhagen, Denmark.

The induction of peroxynitrite generation by Sorafenib plays a relevant role during mitochondrial dysfunction in liver cancer cells (ON-SITE)

Elena Navarro-Villarán[1,2,3], Patricia de la Cruz-Ojeda[1,2,3], Jordi Muntané[1,2,3]

[1]Institute of Biomedicine of Seville (IBiS), Hospital University "Virgen del Rocío"/CSIC/University of Seville, Spain. [2]Biomedical Research Center for Hepatic and Digestive Diseases (CIBERehd), Madrid, Spain. [3]Department of Medical Physiology and Biophysics, University of Seville, Seville, Spain.

Investigating the role of NO in cancer stem cell pathophysiology (ZOOM)

Giasemi Eptaminitaki[1], Benjamin Bonavida[2], Stavroula Baritaki[1]

[1]Laboratory of Experimental Oncology, Division of Surgery, Medical School, University of Crete, Heraklion 71003, Crete, Greece; [2]Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, USA.

ROUND TABLE SESSION 9

11:00-12:00

Round Table (ON-SITE)

NO-based therapeutic strategies in diseases

Dennis J Stuehr

Jean-Luc Balligand

María M. Miranda

Michael T Heneka

Alex Dyson

José M Palma

CLOSING SESSION 10

12:00-13:00

Concluding remarks, awards for the best three communications

Jordi Muntané (Local Organizing Committee, Institute of Biomedicine of Seville)
Lucia Morbidelli (Local Organizing Committee, University of Siena)
Khosrow Kashfi (International Society of Nitric Oxide and Cancer, New York, USA)

The best three communications will be for the presenting authors (predoctoral or postdoctoral researchers under 40 years-old). All three winners will receive the amount of 100 Euros, free registration to the meeting, and one-year ISNOC membership 2022.

13:00-15:00: ***Lunch and farewell of attendees***

KEYNOTE LECTURES, INVITED SPEAKERS AND YOUNG INVESTIGATOR SESSSIONS

Day 1: March 3, 2022

OPENING SESSION I

KEYNOTE LECTURE 1

Moderator: Dr. Ben Bonavida (University of California, USA)

Hemeproteins, NO, and their Relationships in Cancer

Dennis J. Stuehr

Department of Inflammation & Immunology, Lerner Research Institute, Cleveland Clinic, Cleveland Ohio USA

Different aspects of cancer are influenced by several hemeproteins, including the NO synthases, soluble guanylyl cyclase, NADPH oxidases, tryptophan and indoleamine dioxygenases, myoglobin and hemoglobins. In all cases the participation of the hemeprotein relies on it obtaining mitochondrially-generated heme during its maturation to a functional form. The speaker's presentation will provide an update on what is known about intracellular heme trafficking during hemeprotein maturation, including his lab's work in identifying the proteins involved in the heme deliveries, their mechanisms of action, and how the processes may be regulated. Special emphasis will be placed on the cell biology of heme delivery to the tryptophan and indoleamine dioxygenase enzymes, whose heme levels are naturally dynamic and whose activities are thought to help tumors escape from host immune surveillance. A new role for NO in regulating the intracellular heme allocations will also be presented.

SESSION 2

DEVELOPMENT OF NITRIC OXIDE DONORS IN TARGETING DISEASE MANAGEMENT

Moderator: Dr. Lucia Morbidelli (University of Siena, Italy)

INVITED SPEAKERS

Myoglobin regulates metabolism, decreases nitric oxide bioavailability and attenuates proliferation in breast cancer cells

Sruti Shiva

Vascular Medicine Institute and Department of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine

Breast cancer is a leading cause of death in women, and approximately 40% of breast tumors aberrantly express the small monomeric heme protein myoglobin (Mb). Accumulating studies demonstrate that the expression of Mb in breast tumors is associated with better prognosis in humans and decreased tumor burden in animal models of breast cancer. However, the molecular mechanisms by which Mb attenuates tumor growth remains unclear. Altered metabolism and the production of high concentrations of nitric oxide (NO) have been strongly associated with breast cancer tumor aggressiveness. Thus, we hypothesized that Mb, through its NO dioxygenase activity and ability to bind fatty acids, regulates metabolism and decreases NO bioavailability to attenuate breast cancer cell proliferation. Here we utilize MDA-MB-231 triple-negative breast cancer cells that express human Mb (231Mb) and corresponding wildtype cells (231WT) to examine the effect of Mb expression on NO signaling and metabolism. We demonstrate that 231Mb cells show a significantly lower proliferation and migration rate than 231WT cells. Mechanistically, 231Mb cells demonstrate a metabolic switch reflecting increased glucose metabolism and decreased fatty acid oxidation. Further, treatment with NO (Deta-NONOate) increases proliferation rates in 231WT cells and Mb expression significantly attenuates this

increase. Importantly, these protective effects of Mb are when the apo-Mb protein (which lacks the heme moeiety) is expressed. In a murine xenograft model, in which we measured the increase in NOS expression, 231Mb tumors showed decreased weight compared to those without Mb. Together these data implicate Mb-dependent decreased NO bioavailability and regulation of metabolism in attenuation of pro-tumorigenic signaling. Ongoing studies are delineating the exact mechanisms by which Mb regulates metabolic signaling and may offer a potential therapeutic avenue to attenuate tumor progression.

The role of the Arginase-Nitric Oxide pathway in vascular dysfunction and inflammation

Alia Shatanawi

School of Medicine, The University of Jordan, Amman, Jordan

Vascular endothelial dysfunction (VED) is strongly implicated in the pathogenesis of diabetic vascular complications, in atherosclerosis and in a number of inflammatory conditions. Impaired endothelial cell production of nitric oxide (NO) is a main characteristic of vascular dysfunction. In endothelial cells NO is produced by endothelial nitric oxide synthase enzyme (eNOS), by utilizing L-arginine. Arginase in endothelial cells also uses L-arginine as a substrate to produce urea and ornithine. Recently arginase upregulation has been shown to play a role in vascular dysfunction in diabetes by limiting L-arginine bioavailability to eNOS and limiting NO production. Additionally, arginase upregulation has been associated with vascular dysfunction in a number of cardiovascular and inflammatory conditions. These include, but not limited to, atherosclerosis and asthma. Also, arginase is elevated in a number of cancers.

We performed analysis of arginase activity and NO levels in type 2 diabetic patients. Arginase activity was elevated in type 2 diabetic patients while NO production was reduced compared to age-matched healthy volunteers. Levels of arginase activity has a positive correlation with HbA1c levels in diabetic patients. The use of L-citrulline a natural arginase inhibitor resulted in elevation of NO levels in the plasma of diabetic patients while arginase activity was blunted. Cell studies also agreed with these findings as high glucose (25 mmol/L, 72 hrs) treatment to endothelial cells resulted in a 66% increase in arginase activity. This increase in arginase activity was concomitant with a 27% drop in NO produced

by endothelial cells. Inhibitor of arginase (ABH 100 umol/ L) restored NO level to normal. We have additionally identified signaling mechanisms that leads to arginase upregulation and decreased NO production in endothelial cells. Collectively, our results indicate that diabetic conditions cause an elevation of arginase activity which can limit endothelial cells production of NO and thus impair vasorelaxation. Arginase can be regarded as a novel marker for the vascular complications of diabetes. Drugs targeting arginase or its signaling pathway may show benefits in delaying or preventing vascular endothelial dysfunction and in maintaining healthy NO levels.

NO signaling in cardiovascular health and disease

Jean-Luc Balligand

Pole of Pharmacology and Therapeutics, Institut de

Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain (UCLouvain), Brussels, Belgium

Nitric oxide has long been known to exert a crucial function in cardiovascular homeostasis. While substantial knowledge has accumulated on the regulatory mechanisms of NO synthase-NO pathway, the complexity of signaling by this radical gas has been difficult to harness for translation to therapeutics targeted to specific cells of the cardiovascular system. We will review some of the latest paradigms underlying the successes (or failure) of clinical applications of NO-based therapeutics in cardiovascular diseases, as well as highlight the interest in measurements of nitrosylated hemoglobin as a biomarker for diagnostics and treatment tailoring.

YOUNG INVESTIGATOR SESSION

The metal-nonoate Zn(PipNONO)Cl exhibit antitumor activity through inhibition of epithelial and endothelial mesenchymal transitions (EMT and EndMT)

Valerio Ciccone[1,2], Carlotta Pranzo[1], Arianna Filippelli[1], Enrico Monzani3, Lucia Morbidelli[1]

[1] Department of Life Sciences, University of Siena, Siena, Italy; [2] Department of Experimental Medicine, University of Campania “Luigi Vanvitelli”, Naples, Italy; and [3] Department of Chemistry, University of Pavia, Pavia, Italy

Introduction. Nitric oxide (NO) is a ubiquitous, water soluble, free radical gas, which plays key role in various physiological as well as pathological processes. The effect of NO on tumor growth clearly demonstrates its innate bimodal activity, depending on concentration. Indeed, high concentrations (≥0.5 mM) inhibit cell proliferation and invasion and induce apoptosis[1]. Among the NO donors, metal-nonoates are based on the functionalization of a specific polyamine, N-aminoethylpiperazine (Pip), with a “nonoate” group, and stabilized by complexation to a metal ion, i.e. copper(II), nickel(II), and zinc(II). In previously studies, nickel based compound, Ni(SalPipNONO) exhibited an inhibitory activity on lung cancer cells, through the impairment of tumor angiogenesis. Among the developed nonoates, the Zn(II) containing derivatives Zn(PipNONO)Cl exhibited an interesting kinetic of NO release ($t_{1/2}$ 385 s) and a protective effect on vascular endothelium[2].

Aims. We have evaluated the antitumor property of Zn(PipNONO)Cl in human lung cancer cells (A549) in regulating some crucial steps of the metastasis process, beside its antiproliferative capacity. Metastasis initiates with the epithelial-mesenchymal transition (EMT) process consisting in acquisition of invasive and migratory property by tumor cells. Also endothelial-mesenchymal transition (EndMT) helps tumor development through activation of the endothelial/stromal compartment.

Results. At not cytotoxic levels, the nonoate significantly impaired A549 EMT induced by transforming growth factor-β (TGF-β). Reduction of the mesenchymal marker vimentin, upregulated by TGF-β ,and restoration of the epithelial marker E-cadherin, reduced by TGF-β exposure, were detected in the presence of Zn-nonoate. Further, studies were performed to assess the potential role of the NO donor on EndMT, achieved in a tumor-endothelial cell co-culture system. Endothelial cells co-cultured with A549 acquired a mesenchymal phenotype with increased vimentin and reduced VE-cadherin and FGFR-1, typical markers of normal endothelium. The presence in the coculture of Zn(PipNONO)Cl maintained a normal endothelial phenotype with restored VE-cadherin and FGFR-1 expression.

Conclusions. From these in vitro data it results that the metal-nonoate with Zn(II) in the molecule center, appears a promising therapeutic tool to control tumor growth and metastasis acting both on the neoplastic cells and the endothelial component.

References

1. Ciccone V, Monti M, Monzani E, Casella L, Morbidelli L. The metal-nonoate Ni(SalPipNONO) inhibits in vitro tumor growth, invasiveness and angiogenesis. Oncotarget. 2018 Jan 30;9(17):13353-13365. doi: 10.18632/oncotarget.24350. PMID: 29568362; PMCID: PMC5862583. 2. Monti M, Hyseni I, Pacini A, Monzani E, Casella L, Morbidelli L. Cross-talk between endogenous H_2S and NO accounts for vascular protective activity of the metal-nonoate Zn(PipNONO)Cl. Biochem Pharmacol. 2018 Jun;152:143-152. doi: 10.1016/j.bcp.2018.03.025. Epub 2018 Mar 26. PMID: 29588193.

Interactions between Nitric Oxide and Hemin and their Implications in the Nitration of Proteins

Amir Alsharabasy[1], Sharon Glynn[1,2], Pau Farràs[1,3], Abhay Pandit[1]

[1]CÚRAM, SFI Research Centre for Medical Devices, [2]Discipline of Pathology, Lambe Institute for Translational Research, School of Medicine, [3]School of Biological and Chemical Sciences, Ryan Institute, National University of Ireland, Galway

Introduction: •NO interacts with both O_2 and reactive oxygen ions producing different reactive nitrogen species (RNS) involved in the nitration of various functional proteins and contributing to multiple inflammatory diseases and malignant conditions, including breast cancer.[1] Among these, peroxynitrite and mixtures of nitrite and H_2O_2 have been considered critical nitrating species for proteins. Moreover, the nitration reactions induced by nitrite and H_2O_2 were catalyzed by hemin and other iron-porphyrins.[2] However, despite the proven binding affinity between •NO and heme/hemin in different enzymatic systems[3] accompanied with a group of reductive nitrosylation reactions and RNS generation, the possible roles played by •NO and hemin in the nitration of proteins in breast cancer cells have not been explored previously. **Objectives:** 1) To study the interactions between hemin and •NO under the culture conditions of MDA-MB-231 cells and in the presence of bovine serum albumin (BSA). 2) To assess the effects of hemin/•NO-binding on the nitration of intracellular proteins. 3) To understand the mechanism by which hemin and •NO modulate protein nitration, BSA was studied as a model protein. **Experimental design:** the •NO-binding affinity of hemin was evaluated using electrochemical and chemiluminescence-based techniques. Next, the effects of •NO on hemin uptake by MDA-MB-231 triple-negative breast cancer cells were investigated in FBS-containing and FBC-free media, alongside the impact of both on nitration of the intracellular proteins using western blotting. After that, a cell-free assay was developed to study the effects of hemin and •NO on BSA nitration using western blotting with examining the catalytic functions of hemin involved in that process

via a group of spectrometric measurements. **Results:** Hemin was found to have a •NO-binding affinity higher than that of carboxy-PTIO at the same concentration when •NO was delivered from diethylenetriamine NONOate (DETA-NO), accompanied by a decrease in the levels of nitrite in solution. Next, cellular uptake was promoted via hemin nitrosylation, particularly in the FBS-free culture medium compared to the FBS-containing medium. Moreover, it was proved that NO's efficiency to enhance the nitration of the intracellular proteins was improved via the catalytic effects of hemin; however, these nitrating effects were found to be controlled by the concentrations of both hemin and •NO alongside the M.W of the target protein. Similar results were observed in the case of BSA treated with hemin and either DETA-NO or sodium nitroprusside. The protein nitration was dependent on the pH of the solution and concentrations of the reaction components. **Conclusions:** The interactions between hemin and •NO and the products of the nitrosylation reactions were found to play essential roles in inducing the nitration of proteins in breast cancer cells, alongside the already reported reactions. We propose an overall mechanism that can guide us through the development stages of new pharmaceutics to treat various types of cancers. **Acknowledgements:** This work has emanated from research conducted with the financial support of Science Foundation Ireland and is co-funded under the European Regional Development Fund under Grant number 13/RC/2073_P2, the College of Engineering and Informatics, NUIG, Ireland.

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Day 2: March 4, 2022

SESSION 3

NITRIC OXIDE DISTURBANCES IN METABOLIC AND CARDIOVASCULAR DISORDERS

Moderator: Dr. Sharon Glynn (National University of Ireland, Ireland)

INVITED SPEAKERS

Engineering Polymeric NanoParticles for Advanced Applications

Cyrille Boyer

Centre for Advanced Macromolecular Design (CAMD); Australian Centre for NanoMedicine (ACN); School of Chemical Engineering, The University of New South Wales, Sydney, NSW 2052, Australia

Soft core-shell polymeric nanoparticles are an area of great research interest, due to their potential advantages in the sustained and targeted delivery of therapeutic payloads. These systems can offer significant improvements in the temporal and spatial control of drug delivery. In this talk different polymeric nanoparticles that have been specifically designed to deliver anti-cancer drugs and to image specific tissue, will be discussed. The first system presented will be based on pH- and redox- responsive nanoparticles which are able to deliver different payloads in different cellular compartments. The synthesis and the characterization of these nano-objects will be outlined in detailed. As an example, the delivery of nitric oxide will be presented using these nanoparticles for the treatment of liver fibrosis and neuroblastoma. We have also demonstrated synergistic effect when we combine nitric oxide (NO) with chemotherapy drugs for the treatment in multi-drugs resistance in cancer. In a second part of this talk, the synthesis of new hybrid organic/inorganic nanomaterials, based on iron oxide, gold and gadolinium, will be reported for use as MRI contrast agents. The effect of the architecture and the nature of polymers will be correlated with the magnetic properties of these nano-objects. In addition, the polymeric shell of these nanomaterials can be designed to conjugate with anti-cancer drugs. Finally, I will rapidly mention the use of hybrid inorganic polymeric nanoparticles for the storage of hydrogen. In this part, I will present and discuss on the synthesis of magnesium hydride (MgH_2)

nanoparticles stabilized and assembled using functional polymer to yield a new generation of nanomaterials with remarkable hydrogen storage properties.

Photoresponsive polypeptide nanomedicines: from rational design to efficient NO gas delivery and cancer therapy

Chang-Ming Dong

School of Chemistry & Chemical Engineering, Shanghai Jiao Tong University, Shanghai 200240, P. R. China

Stimuli-responsive polymeric nanomedicines that respond to external and physiological stimuli such as temperature, pH, light, redox, etc., can enhance the drug efficacy and realize on-demand drug release and dosing in target sites, holding potential for clinical medicine. Because of their inherent ionizable groups (e.g., amine and carboxy groups), hierarchical self-assembly and multiple secondary and/or tertiary structures, biodegradable polypeptides have been intensively designed and fabricated into stimuli-responsive (e.g., pH and temperature) nanomedicines for drug/gene/siRNA and protein delivery [1, 2]. In the past years, we focused on the design of external light-responsive polypeptides and their nanomedicine applications. I will give some examples to discuss the light-responsive polypeptide self-assemblies for controlled drug release, the sugar-targeted polypeptide nanoparticles, the polypeptide nanomedicines for the combined photothermal and (cocktail) chemotherapy (i.e., PTT-CCT and PTT-CT), and the polypeptide nanocomposites for the triple therapies of PTT, NO gas therapy, and chemotherapy (i.e., PTT-NO-CT). In vivo PTT-NO-CT treatment achieved superior anticancer efficacy with 1+1+1 > 3 effect, opening up new avenue for reversing cancer multidrug resistance[3-6].

Acknowledgments. Financial support by National Natural Science Foundation of China (22075176) and Natural Science Foundation of Shanghai (22ZR1429200) is greatly appreciated.

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Targeting dimethylarginine dimethylaminohydrolase 1 to suppress vasculogenic mimicry in cancer: current evidence and future directions

Arduino A Mangoni

Discipline of Clinical Pharmacology, College of Medicine and Public Health, Flinders University, Adelaide, Australia; Department of Clinical Pharmacology, Flinders Medical Centre, Southern Adelaide Local Health Network, Adelaide, Australia

Introduction: The isoform 1 of dimethylarginine dimethylaminohydrolase (DDAH1) metabolizes the endogenous nitric oxide (NO) synthase inhibitors asymmetric dimethylarginine (ADMA) and monomethyl arginine (L-NMMA) to L-arginine and dimethylamine. Increased DDAH1 expression has been recently linked to the capacity of cancer cells, particularly triple negative breast cancer (TBNC), to form blood vessel-like structures, a phenomenon called vasculogenic mimicry (VM). VM plays a critical pathophysiological role in breast cancer and other cancers as its presence predicts cancer aggressiveness, metastasis, and poor survival.

Aims: We investigated the effects of two arginine analogues synthesized by our group with inhibitory activity towards DDAH1, ZST316 (IC_{50}: 3μM; K_i: 1μM) and ZST152 (IC_{50}: 18μM; K_i: 7μM), on VM in the TNBC cell lines MDA-MB-231 and BT549. A UPLC-MS assay to quantify stability and intracellular concentration of the DDAH1 inhibitors was also developed.

Results: In an *in vitro* assay of VM, both ZST316 (Figure 1) and ZST152 significantly attenuated VM in a dose-dependent fashion. This effect was not due to cell toxicity or altered cell proliferation but might be due in part to inhibition of cell migration. At all concentrations and timepoints assessed, there was no significant degradation of the compounds from the culture media. The concentrations of ZST316 and ZST152 in the assay cell lysates, 0.32 µM and 0.28 µM, translated into an intracellular concentration of 39 and 35 pmol/million cells, respectively. Mechanistically, we observed a significant modulation of the DDAH/ADMA/NO pathway following exposure of 100 µM ZST316 or ZST152: a 40% increase in the DDAH1 substrate ADMA, and a 38% decrease in the DDAH1 product L-citrulline.

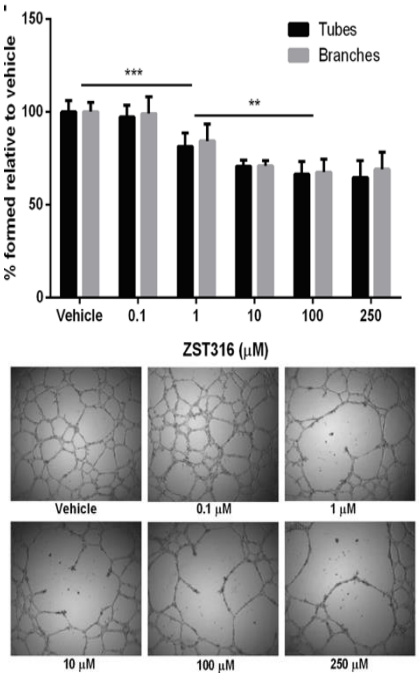

Figure 1. Effect of ZST316 on the number of tubes and branches formed by TNBC cells (VM).

Conclusions and future directions: This study represents the first evidence for therapeutic inhibition of DDAH1 by small molecules in breast cancer. More recently, we have a) observed similar anti-VM effects in TNBC cells with another DDAH1 inhibitor synthesized by a collaborator, and b) demonstrated that ZST316 possesses a favorable pharmacokinetic profile and excellent tolerability during chronic treatment in mice. In vivo studies investigating the effects of ZST316 in xenograft models of TNBC are being planned.

YOUNG INVESTIGATOR SESSION

Nitric oxide as an activator of HER2 in breast cancer

Ciara O'Neill, Eoin Dervan, Jake McAuliffe, Suguna Sundararaman, Sharon Glynn

Discipline of Pathology, Lambe Institute for Translational Research, National University of Ireland Galway.

Hypothesis: HER2 receptor signaling is activated by nitric oxide in breast cancer.
Introduction: In Ireland over 3000 women are diagnosed with breast cancer annually, of these tumors approximately 20% overexpress the HER2 protein. HER2 is a known driver of proliferation and metastasis in breast cancer. Various therapeutics targeting HER2 have been developed. High levels of inducible nitric oxide synthase (iNOS) expression have been linked with poor outcomes in ER- breast cancer. One mechanism is through s-nitrosylation of EGFR. In this study

we explore where nitric oxide activates the HER2 receptor similar to its sister receptor EGFR. **Aims:** Firstly, to assess NO's effect on HER2 phosphorylation. Secondly, to verify HER2 activation by NO induces downstream signaling. **Experimental design:** HCC1954 HER2 amplified breast cancer cells were serum starved for 24 hours prior to treatment with 300µM and 500µM DETA/NO alongside a vehicle control. Cell lysates were isolated at various timepoints for analysis by Western blot. **Results:** The HER2 receptor undergoes phosphorylation at the tyrosine 1221/1222 residue within 1 hour and 6 hours following treatment with 300µM and 500µM DETA/NO respectively. Rapid turnover of the HER2 receptor following activation was also seen. **Conclusions:** Nitric oxide is capable of activating the HER2 receptor and triggering downstream signaling cascades.

Oxidative stress-induced endothelial dysfunction and decreased vascular nitric oxide in COVID-19 patients

Virginie Montiel[1,2], Irina Lobysheva[2], Ludovic Gérard[1,3], Marjorie Vermeersch[4], David Perez-Morga[4], Thomas Castelein[1], Jean-Baptiste Mesland[1], Philippe Hantson[1], Christine Collienne[1], Damien Gruson[5], Marie-Astrid van Dievoet[5], Alexandre Persu[6,7], Christophe Beauloye[6,7], Mélanie Dechamps[1,7], Leïla Belkhir[8], Annie Robert[9], Marc Derive[10], Pierre-François Laterre[1], A.H.J Danser[11], Xavier Wittebole[1], Jean-Luc Balligand[2]

[1]Intensive Care Unit, Cliniques Universitaires Saint-Luc, Brussels, Belgium; [2]Institute of Experimental and Clinical Research, Pole of Pharmacology and Therapeutics, Université catholique de Louvain, Brussels, Belgium; [3]Institute of Experimental and Clinical Research, Pole of Pneumology, ENT and Dermatology, Université catholique de Louvain, Brussels, Belgium; [4]Center for Microscopy and Molecular Imaging, Université Libre de Bruxelles, Gosselies, Belgium; [5]Department of Laboratory Medicine, Cliniques Universitaires Saint-Luc, Brussels, Belgium; [6]Institute of Experimental and Clinical Research, Pole of Cardiovascular Research, Université catholique de Louvain, Brussels, Belgium; [7]Department of Cardiology, Cliniques Universitaires Saint-Luc and Université catholique de Louvain, Brussels, Belgium; [8]Department of Internal Medicine, Cliniques Universitaires Saint-Luc and Université catholique de Louvain, Brussels, Belgium; [9]Institute of Experimental and Clinical Research, Pole of Epidemiology and Biostatistics (EPID), Université catholique de Louvain, Brussels, Belgium; [10]Inotrem SA, Vandoeuvre-les-Nancy France, France; [11]Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands

Objectives/Hypothesis: SARS-CoV-2 targets endothelial cells through the angiotensin-converting enzyme 2 receptor. The resulting endothelial injury induced widespread thrombosis and microangiopathy. Early specific markers of

endothelial dysfunction and vascular redox status in COVID-19 patients are currently missing. **Experimental design:** We prospectively compared clinical and biological parameters in 30 ICU- and 30 non-ICU-admitted COVID-19 patients, 15 matched control subjects for similar cardiovascular risk factors as ICU COVID-19 and 10 ICU-admitted patients with septic shock unrelated to COVID-19. **Results:** Early SARS-CoV-2 infection was associated with an imbalance between an exacerbated oxidative stress (plasma peroxides levels in ICU patients vs. controls: 1456.0 ± 400.2 vs 436 ± 272.1 mmol/L; P< 0.05) and a reduced nitric oxide bioavailability (5-α-nitrosyl-hemoglobin, HbNO) proportional to disease severity (HbNO in ICU patients vs. controls: 116.1 ± 62.1 vs. 163.3 ± 46.7 nmol/L; P< 0.05). HbNO levels correlated with oxygenation parameters (PaO_2/FiO_2 ratio) in COVID-19 patients (R^2 =0.13; *P*< 0.05). Plasma levels of angiotensin II (ICU patients vs. controls: 1.2 ± 1.0 vs. 2.0 ± 1.1 fmol/m; P< 0.05), aldosterone (235.1 ± 145.1 vs. 284.4 ± 181.4 pg/mL; P=NS), renin (20.0 ± 22.4 vs 19.7 ± 13.2 pg/mL; P=NS) or serum level of TREM-1 (ICU patients vs. ICU septic shock unrelated to COVID-19: 265.3 ± 161.2 vs 608 ± 338.3 pg/mL; P< 0.05) ruled out any hyper-activation of the renin-angiotensin-aldosterone system or the leucocyte respiratory burst in ICU COVID-19 patients, contrary to septic patients. Electron microscopy illustrated irregular aspect of the endothelial wall due to fibrillar network of fibrin depots and damaged but viable endothelial cells responsive to circulating autacoids. **Conclusions**: Endothelial oxidative stress with ensuing decreased NO bioavailability appears as a pathogenic factor of endothelial dysfunction in ICU COVID-19 patients. A correlation between NO bioavailability and respiratory parameters is observed in hospitalized COVID-19 patients. These results highlight an urgent need for oriented research leading to a better understanding of the specific endothelial oxidative stress that occurs during SARS-CoV-2.

SESSION 4

NITRIC OXIDE IN HYPERTENSIVE-RELATED DISEASES

Moderator: Dr. Stéphanie Plenchette (EPHE PSL-University of Burgundy, France)

INVITED SPEAKERS

Advances in the discovery of novel agents for the treatment of glaucoma

Claudiu T. Supuran

Università degli Studi di Firenze, NEUROFARBA Department, Sezione di Scienze Farmaceutiche e Nutraceutiche, Via Ugo Schiff 6, 50019 Sesto Fiorentino (Florence), Italy.

Glaucoma, a neuropathy characterized by increased intraocular pressure (IOP) is the major cause of blindness worldwide and its treatment aims at reducing IOP. The drug design for the main classes of anti-glaucoma agents with emphasis on those incorporating NO-donors will be presented [1,2]. Drugs which interfere with the aqueous humor secretion (adrenergic agonists/antagonists, carbonic anhydrase inhibitors) and with its outflow, by means of both conventional and non-conventional pathways (prostaglandin (PG) analogs, rho kinase inhibitors, nitric oxide (NO) donors) as well as new agents (adenosine receptors modulators, melatonin - fatty acid amide hydrolase hybrids, tyrosine kinase activators, natriuretic peptide analogs) will be discussed [3-7]. The anti-glaucoma drug field experienced relevant developments in the last years with the approval of several new drugs belonging to novel pharmacological classes, such as the rho kinase inhibitors ripasudil and netarsudil, and the PG-NO donor hybrid latanoprostene bunod. Eye drops with combinations of two different drugs are also available, allowing for an effective IOP control, with once daily administration for some of them, which assures a better patient compliance and ease of administration. The last year afforded thus interesting new pharmacological opportunities for the management of this disease, with the NO donating agents playing a relevant role [1,2,8].

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The value of sulfur nutraceuticals for the cardiovascular inflamm-ageing: possible role of hydrogen sulfide

Alma Martelli

Department of Pharmacy, University of Pisa, Pisa, Italy

Sulfur nutraceuticals (or sulfaceuticals®) represent all the edible sources (mainly plant extracts from *Alliaceae* and *Brassicaceae*) containing molecules able to release hydrogen sulfide (H_2S), both directly or through their metabolites. Growing evidence converge to support the hypothesis that the cardiovascular (CV) effects induced by sulfur nutraceuticals are due to their property to be an exogenous source of hydrogen sulfide (H_2S). H_2S, previously known only as toxic gas with a typical smell of rotten eggs, is now widely recognized as the third endogenous gasotransmitter, beside nitric oxide (NO) and carbon monoxide (CO) and, like NO and CO, it is fundamental for the maintenance of homeostasis in several districts[1]. As concerns the mechanism of action, H_2S, according to its chemical feature, exhibits anti-oxidant properties, but it acts also through specific mechanisms like the activation of several types of ion channels, such as the ATP-sensitive (KATP)[2]- or the voltage-dependent (Kv7)-potassium channels[3] and through the inhibition of the phosphodiesterase[4]. The molecular mechanism

of sulfhydration of target proteins (i.e. K^+ channels and enzymes), inducing conformational changes, could account for almost all the numerous biological effects of H_2S. At CV level, H_2S induces lowering of blood pressure, cardioprotection, antiplatelet property and anti-inflammatory effect against the vascular inflammation.

Polysulfide deriving from *Alliaceae* (e.g. garlic or onions) and isothiocyanates deriving from *Brassicaceae* (e.g. broccoli, kale or rocket) are exogenous sources of H_2S. The CV effect of garlic was well-known in the ethno-pharmacology and only recently its ability to release H_2S has been demonstrated. As concerns broccoli or rocket, first we demonstrated their features as H_2S-donors and then we evaluated their CV impact. In particular, in last years, our group focused its research interest on the CV evaluation of an isothiocyanate, erucin, deriving from *Eruca sativa* Mill., also called arugula or rocket salad.

The CV effect of erucin has been tested both *in vitro* on human umbilical vein endothelial cells (HUVEC) or on human aortic smooth muscle cells (HASMC), *ex-vivo* on rat isolated aortic rings, and *in vivo* on normotensive and spontaneously hypertensive rats. As result of these studies, erucin resulted as a real H_2S-donor endowed with hyperpolarizing, vasorelaxing, and anti-hypertensive effects[5]. Moreover, erucin significantly prevented the endothelial harm induced by high concentrations of glucose, preserving the cell viability, inhibiting the increase of oxidative and pro-inflammatory markers and reducing the increase of endothelial hyperpermeability[6].

In conclusion, erucin, the isothiocianate deriving from *Eruca sativa* Mill., showed protective CV effects against vascular inflammation, endothelial dysfunction and hypertension. These properties could be due to its ability to release H_2S and pave the way for a potential use of sulfur nutraceuticals in the management of CV disease.

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Nitric oxide roles in retinal pathologies

María M. Miranda

Departamento Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Cardenal Herrera-CEU, CEU Universities, 64315 Valencia, Spain

Retinal nitric oxide (NO) functions are extremely important as it is involved in the visual transduction cascade and in retinal blood flow control. However, NO may also be deleterious to the retina. Retina is rich in polyunsaturated fatty acids and is exposed to great oxygen concentrations. Therefore, NO can easily generate dinitrogen trioxide and peroxynitrite, with important harmful consequences.

Herein, we review NO role in three different retinal degenerations: diabetic retinopathy (DR), age-related macular degeneration (AMD) and retinitis pigmentosa (RP).

DR has been classically considered a microvascular disease but increasing evidence suggests it is also a neurodegenerative disease. In diabetic patients increased NO plasma concentrations are associated with increased diabetic retinopathy severity. Several nitric oxide synthase (NOS) inhibitors prevent the changes induced by diabetes in rats.

AMD is the leading cause of blindness in the elderly in developed countries. A vascular theory (that includes abnormalities of the choroidal circulation) and the involvement of oxidative stress and NO has been suggested to contribute to AMD development. It has been shown that nNOS and eNOS expression decreased in AMD eyes, when compared to control patients. Other researchers have suggested an increase in NO production in AMD patients.

RP is a group of retinal hereditary pathologies that cause photoreceptor cell death. Our research has observed alteration of NOS in three different RP animal models (the rd1, rd10 and rds mice). We also demonstrate that treatment with molecules that are known to be NO inhibitors (such as progesterone or dutasteride) restore NOS alteration and delay photoreceptor death.

YOUNG INVESTIGATOR SESSION

Excessive *S*-nitrosylation induced by GSNOR deficiency increases malignancy in rhabdomyosarcoma

Costanza Montagna[1], Chiara Pecorari[2], Paola Giglio[1], Valeria Fiorentini[1], Salvatore Rizza[2], Giuseppe Filomeni[1,2,3]

[1]Department of Biology, University of Rome Tor Vergata, Rome, Italy. [2]Redox Biology Group, Danish Cancer Society Research Center, Copenhagen, Denmark. [3]Center for Healthy Aging, University of Copenhagen, Denmark.

Rhabdomyosarcoma (RMS) is the most common pediatric mesenchymal malignancy with poor prognosis, underscoring an urgent need for therapy. Targeted therapy is largely used in RMS clinical trials; however, the effect of the targeted therapy as a single treatment is modest in patients with advanced metastatic RMS. Therefore, it is essential to develop novel strategies to be used with the standard of care as an adjuvant approach. GSNOR is the nitric oxide (NO)- detoxifying enzyme and displays many features of a prototypic tumor suppressor by regulating the quality and the quantity of *S*-nitrosylated proteins. In silico analyses from the TGCA database show that GSNOR is significantly downregulated in human sarcomas.

Additionally, GSNOR is a critical enzyme in the progression through myoblast differentiation into myotubes, and RMS arises from neoplastic primitive muscle cells (myoblasts) that fail to differentiate into myotubes. Accordingly, preliminary data indicates GSNOR suppression inhibits RMS cells myogenic differentiation, increases RMS cells malignancy, and causes mitochondrial impairment. In the current project, I propose investigating how S-nitrosylation induced by GSNOR deficiency results in i) increased proliferation, ii) mitochondrial impairment, iii) metastatic phenotypes. Finally, I will test if the vulnerabilities of cancer cells triggered by GSNOR deficiency can be used to tailor a targeted adjuvant therapy for RMS treatment.

Unraveling the role of AKR1A1 in renal and hepatocellular carcinoma progression

Chiara Pecorari[1], Giuseppe Filomeni[1,2]

[1]Redox Biology Group, Danish Cancer Society Research Center, Copenhagen, Denmark. [2]Department of Biology, University of Rome Tor Vergata, Rome, Italy

Renal and hepatocellular carcinoma (RCC and HCC, respectively) are two of the most worldwide common causes of death, and their incidence have risen steadily. Both RCC and HCC have been reported to undergo a metabolic reprogramming that plays a major role in tumor development. A promising field

in cancer treatment is targeting the processes that cause these metabolic changes. Among these processes, the protein modification induced by nitric oxide (NO) – so-called S-nitrosylation – has recently been proposed to be involved in metabolic adaptation. In agreement, loss of the denitrosylase aldo-ketoreductase 1A1 (AKR1A1), an enzyme that preserves protein from undergoing S-nitrosylation, has recently been reported to rewire kidney metabolism. With this in mind, we went through The Human Protein Atlas database and observed that HCC and RCC are characterized by low AKR1A1 expression. In silico analysis performed in our laboratory further show a correlation between AKR1A1 hypo-expression and poor prognosis of RCC patients. Based on these results, we hypothesize that AKR1A1 has a role in HCC and RCC development. By integrating cellular and molecular approaches with high-throughput analyses, we found out that AKR1A1-downregulation induces a wide metabolic reprogramming in RCC and HCC cell lines that results in: (i) lower glycolytic rate, (ii) boosted pentose phosphate pathway and (iii) increased levels of methylglyoxal (MGO), a side-product of glycolysis. High MGO levels have been correlated with advanced glycation end-products (AGEs) formation, Nrf2-activation and induction of epithelial-to-mesenchymal transition (EMT). Coherently, our results show that AKR1A1-deficient RCC and HCC cell lines activate (i) Nrf2 and antioxidant response, (ii) EMT and (iii) increased resistance to chemotherapeutics. Altogether, our results suggest a role of AKR1A1 in the redox adaptation response and in the acquisition of an invasive and aggressive phenotype of HCC and RCC. Unraveling the molecular mechanism underlying these phenotypes will help provide new therapeutic targets to treat advanced RCC and HCC and improve patient survival.

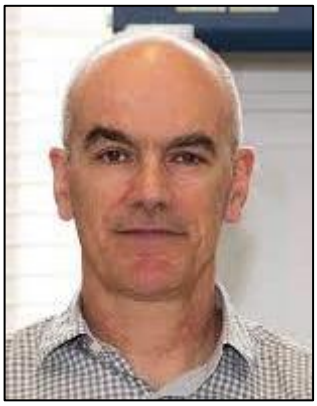
SESSION 5

NITRIC OXIDE AND NEURODEGENERATION

Moderator: Dr. Giuseppe Filomeni (Danish Cancer Society Research Center, Denmark)

INVITED SPEAKERS

Modulation of neurovascular in the brain by nitric oxide and cognitive enhancement



João Laranjinha[1,2], Cátia Lourenço[2], Ana Ledo[1,2], Carla Nunes[2], Ricardo Ferreira[2], Cândida Dias[2], João Gonçalves[2] and Rui Barbosa[1,2]

[1]Faculty of Pharmacy and [2]Center for Neurosciences and Cell Biology, University of Coimbra, Portugal

Neuronal activity imposes a need for blood flow-carrying substrates in order for the brain to maintain its functional and structural integrity. The fine temporal and spatial adjustment of cerebral blood flow (CBF) to the metabolic demands of active neurons is termed neurovascular coupling, NVC (or functional hyperemia). The NVC is largely driven by glutamate release, but a great uncertainty pertains to the molecular mechanisms that bridge increases of neuronal activity with local regulation of CBF.

By developing innovative tools for in vivo assessment of NVC we have described neuronal nitric oxide (NO), along the NMDAreceptor-nNOS-NO pathway, as direct mediator of the communication between neurons and local microvessels (Lourenço et al 2014). The functionality of NVC is key for cognitive performance and becomes impaired during aging and age-associated neurodegeneration, notably Alzheimer´s disease (Lourenço et al. 2017). Considering the nitrate:nitrite:NO pathway and that nitrite might be univalently reduced to NO by biological reductants, acting as a biological precursor of NO, we and others have come to conjecture that the redox and functional interplay of nitric oxide with ascorbate and nitrite would modulate the functionality of glutamatergic synapses in terms of NVC.

We have used a multimodal approach, comprising microarrays for stereotaxic insertion in the brain of living rodents, consisting of microinjection pipettes, laser Doppler blood flow probes and selective microelectrodes, behavior and biochemical approaches to probe the dynamics and functional impact of O_2, glucose, NO, ascorbate and CBF in vivo in terms of NVC in animal models of aging and AD disease.

Data in vivo in rodents supports that (1) the redox interaction of nitrite/ascorbate/NO functionally coupled to neuronal activation supports neurovascular coupling, 2) rescue from impaired neurovascular coupling results in enhancement of cognitive performance.

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Inflammation in neurodegenerative disease: Role of iNOS and nitric oxide

Michael T Heneka

Luxembourg Centre for Systems Biomedicine-LCSB, University of Luxembourg, Luxembourg

Neurodegenerative disease including Alzheimer's disease are characterized by an inflammatory component along the disease trajectory. Part of this neuroinflammatory challenge to the CNS is the induction of the inducible isoform of nitric oxide synthase (iNOS/NOS2) that results in prolonged release of iNOS-derived NO by microglia, macrophages and astrocytes. While low and transient production of nitric oxide plays a number of physiologically important roles,

sustained release of NO may also have harmful effects. Such, iNOS derived NO has been shown to affect long-term potentiation in the murine hippocampus, a memory consolidating step during long-term information storage. In keeping with this we investigated the role of iNOS derived NO for NO-mediated post-translational modification of synaptic proteins, in synaptosome preparations from APP/PS1 transgenic mice and human AD cases. Both data sets provide evidence for an NO-mediated change of synaptic proteins. In testing how much such a NO—mediated post translational modification can change peptide or protein function we investigated the nitration of beta-amyloid itself at its tyrosine located at position 10. Nitration strongly increased Abeta's propensity to aggregate and made it also harder to degrade and digest for microglia. In in vivo seeding models of cerebral amyloidosis, NO-mediated nitration of the beta-amyloid peptide increased overall deposition.

Together, these data suggest that NO may also exert harmful effects during the course of neurodegenerative disease. Its use as a therapeutic or its inhibition should be evaluated with a close look on the exact time point and site of action.

NO-mediated neuroinflammatory pathways as treatment targets in neurodegeneration.

Joern R Steinert

Division of Physiology, Pharmacology and Neuroscience. Faculty of Medicine and Health Sciences, University of Nottingham, UK

Numerous neurodegenerative diseases associated with protein misfolding (e.g. Alzheimer's and Parkinson's disease) exhibit enhanced oxidative and nitrergic stress conditions following initiation of neuroinflammatory pathways. The underlying activation of microglia within the central nervous system is responsible for the release of pro-inflammatory signaling molecules and the associated release of nitric oxide (NO) which provides a potent contribution to cytotoxic redox signaling. NO-mediated post-translational protein modifications impact upon protein functions and can exacerbate pathological processes. In addition, non-enzymatic and irreversible glycation signaling has been implicated as an underlying pathway that promotes protein misfolding via the generation of advanced glycation end-products (AGE). Following activation of specific receptors recognizing AGEs (RAGE) further oxidative stress and production of

cytokines induces an upregulation of inflammatory mediators. However, the direct interactions between both, NO-mediated neuroinflammation and RAGE signaling, pathways are poorly understood.

We investigated the therapeutic potential of suppressing NO signaling during early progression of prion disease. Prion misfolding induces hippocampal gene and protein expression of oxidative and nitrergic stress markers. We analyzed electrophysiological characteristics of pyramidal hippocampal neurons in diseased and control mice detailing the neurodegeneration during early time points. To study the impacts of NO during the pathology, mice were injected with a NO synthase (NOS) inhibitor at early disease stages and the time course of disease markers was assessed over the crucial age range of early pathology.

Increased neuroinflammatory signaling was observed in mice between 6 and 10 weeks post inoculation (w.p.i.) with scrapie prion protein which was characterized by enhanced nitrergic stress and associated with a decline in hippocampal neuronal function by 9 w.p.i.. Daily *in vivo* administration of the NOS inhibitor L-NAME between 6 and 9 w.p.i. at 20 mg/kg prevented the functional degeneration of hippocampal neurons in prion-diseased mice. We further found that this intervention in diseased mice reduced 3-nitrotyrosination of triose-phosphate isomerase (TPI), an enzyme involved in the formation of disease-associated glycation and AGE formation. Furthermore, L-NAME application reduced the degree of TPI nitrotyrosination and the expression of the receptor for advanced glycation end products (RAGE). This work concludes that NO mediated post-translational modifications of TPI may enhance glycation signaling which contributes further to cytotoxicity and accumulation of misfolded prion proteins and thus illustrates an interaction between glycation and NO signaling.

SESSION 6

ACUTE AND CHRONIC INFLAMMATORY-RELATED DISEASES

Moderator: Dr. Khosrow Kashfi (The City College of New York, USA)

INVITED SPEAKERS

NOS and COX Inhibition Augments Immune Polarization and Improves Survival in Radiotherapy: Are NSAIDs and NOS inhibitors a New Approach to Immunotherapy?

Lisa A Ridnour

National Cancer Institute, Frederick, USA

Immune therapy has emerged as the new frontier of cancer treatment. Therapeutic radiation is a known inducer of immune response that can be limited by immunosuppressive mediators including cyclooxygenase-2 (COX2), which is highly expressed in triple negative breast tumors. Importantly, a clinical cohort of triple negative breast cancer patients revealed that elevated COX2 tumor expression predicted poor radiation therapeutic efficacy. Moreover, this study shows that treatment with ionizing radiation and adjuvant NSAID (indomethacin) therapy, which inhibits COX2 activity provides a powerful combination to reduce both primary tumor growth and lung metastasis through improved immune polarization. By employing a unique multiplex imaging platform consisting of CODEX, Ultivue IHC, Imaging Cytoff and RNAscope microscopic imaging technologies, this work reveals specific spatial immunological changes in the treated primary tumor. Both whole tumor RNA_{seq} and microscopic imaging show that indomethacin alone as well as radiation/indomethacin combination changes the lymphoid population in the primary tumor as defined by increased $CD45^+$ cells and $CD8^+/CD4^+$ spatial ratio coverage throughout the tumor. This combination induces Type 1 IFN, which improves lymphoid infiltration into the tumor epithelium. In addition, the radiation-indomethacin combination suppresses key immune checkpoints including PD1 and CTLA4, which dramatically increased B cell and T cell populations with increased active $CD8^+$ T cells and reduced T cell exhaustion. Thus, adjuvant NSAID treatment in

combination with radiation therapy shifts immune desert phenotypes to proinflammatory immune phenotypes favoring N1/M1/Th1 signatures in an immunologically challenging tumor model. Importantly, radiation-indomethacin combination improved local control of the primary lesion and reduced metastatic burden, which increased the median survival on combination-treated mice when compared to mice treated with single agent radiation. These results suggest that clinically available NSAID's augment radiation therapeutic efficacy and offer a novel approach for combination immunotherapy.

Nanomedicine allied to nitric oxide donors in cancer: Where are we and where can we go?

Amedea B Seabra

Nanomedicine Research Unit, Federal University of ABC, Santo André, Brazil

The free radical nitric oxide (NO) is a signaling molecule controlling important biological processes. Depending on its concentration, location and cellular environment, NO can have protective or toxic effects. Several classes of NO donors/generators have been prepared and combined with nanomaterials, in particular, with polymeric nanoparticles. Engineered nanoparticles (polymeric nanoparticle and metal/metal oxide nanoparticles) are attractive nanocarriers extensively used in biomedical applications, particularly, in cancer biology due to their ability to promote a site-target therapeutic effect, with minimum side effects to health tissues. NO-releasing engineered nanoparticles can have direct toxic effects on tumor cells, or it can promote cancer cell sensitization for traditional cancer treatments. The combination of NO-releasing nanoparticles with conventional anticancer therapies is a promising approach in the reversion of multidrug resistance (MDR) cells. This presentation highlights and discusses the recent progress in the cytotoxicity (tumoral and non-tumoral cell lines) of NO-releasing nanomaterials and the *in vivo* biocompatibility of NO-releasing nanoparticles. Moreover, the ability of these nanoparticles to combat MDR, their mechanisms of toxicity and drawbacks are also discussed.

Nitric Oxide in Macrophage Immunometabolism: Hiding in Plain Sight

Daniel W McVicar

National Cancer Institute, Frederick, USA

The tumor microenvironment represents a complex, multicellular milieu, where various cell types compete for resources necessary for their function(s). In the case of the tumor, cells must rapidly reproduce while promoting expansion of the host vasculature to maintain nutrient supplies. At the same time, tumor cells subjugate host defense mechanisms to thwart rejection. Many mechanisms for tumor-mediated immunosuppression have been described, and several involve mechanisms based on metabolic alterations. For example, highly glycolytic tumors deplete glucose in the microenvironment suppressing T cell proliferation and activation. The resulting elevated lactate from the tumor can transcriptionally reprogram tumor-associated macrophages into immunosuppressive cells promoting tumor growth and progression. The talk is focused on the expression, signaling and function of receptors expressed by innate immune cells in the context of cancer. A variety of receptor systems including the triggering receptors expressed on myeloid cells (TREM) that regulate neutrophils, macrophages, monocytes, dendritic cells and platelets, and the killer Ig-like receptors (KIRs) regulating natural killer (NK) cells. In recent years, interest in understanding stimulation-induced alterations in cellular metabolic processes and the critical role that they play in enabling immune cells to meet the enhanced metabolic demands associated with activation has exploded. Immunometabolism presents exciting possibilities for unraveling, and possibly therapeutically exploiting, the substantial metabolic interactions between inflammatory cells and the tumor. Therefore, we present data showing the dissection of the biochemical mechanisms underlying activation-induced metabolic changes in innate immune cells.

SESSION 7

KEYNOTE LECTURE 2

Moderator: Dr. Jordi Muntané (Institute of Biomedicine of Seville, Spain)

NO-based therapeutic intervention in cancer

David A Wink

National Cancer Institute, Frederick, USA

Over the last decade, the role of free radicals and oxidative stress has been shown to be important in a vast number of biological processes. The diatomic molecule nitric oxide (NO) has been shown to participate in a large number of physiological processes ranging from cardiovascular and neurologic to playing essential roles in a variety of immunological responses. This broad range of biologic effects has created one of the fastest growing fields in biomedical science. With this in mind, we have sought to determine if there are roles for this diatomic radical in cancer treatment.

Nitric oxide participates in various processes associated with cancer biology. Though NO possesses tumoricidal properties, it also can promote tumor growth. Our research is aimed at understanding the role of NO with different processes in tumor biology at the chemical, biochemical, cellular, and physiological levels to explore potential new strategies. NO and other small redox-reactive molecules promote the progression of human cancers. Our research goals are focused on the identification of redox-related mechanisms and biomarkers expressed during chronic inflammation as it relates to cancer progression and poor clinical outcome.

The major determinant for NO effects *in vivo* is its chemical properties. The chemistry of NO in biological systems is complex, and each reaction has potential deleterious or beneficial effects. To decipher the chemical reactions that may account for the paradoxical effects of NO, we have developed a discussion referred to as the chemical biology of NO, which is a guide to the chemistry of NO that can take place *in vivo*. This compilation of chemical reactions provides the fundamental information needed to translate the chemistry of NO into methods for improving modalities of cancer treatment.

Day 3: March 5, 2022

SESSION 8

PROTECTIVE EFFECTS OF NUTRACEUTICALS: FOCUS ON NITRIC OXIDE AND OTHER GASOTRANSMITTERS

Moderator: Dr. Valentina Rapozzi (University of Udine, Italy)

INVITED SPEAKERS

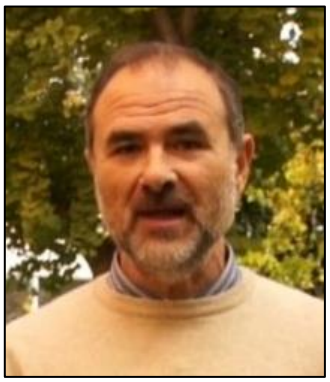
Selenium and hydrogen selenide: essential micronutrient and the fourth gasotransmitter?

Alex Dyson

Institute of Pharmaceutical Science, and Centre for Pharmaceutical Medicine Research, King's College London, London, United Kingdom.

Two decades ago, hydrogen sulfide was included as the third endogenous gasotransmitter after nitric oxide and carbon monoxide. Inclusion into this class requires agreement on five key characteristics including the gaseous nature of these molecules, their production and transport within the body, and mechanistic knowledge on their defined function(s) and specific molecular target(s). Over two hundred years ago, Jons Jakob Berzelius discovered elemental selenium and commented on its similarity to sulfur. Selenium is now known as an essential micronutrient that is incorporated into 25 'selenoproteins'. Within this canonical biological framework, the majority of these proteins act as oxidoreductase enzymes that are crucial for detoxification of reactive oxygen species. However, given the proximity of sulfur and selenium in the periodic table, these elements (and their physiological derivatives), share many chemical and biological characteristics. As such, we and others have hypothesized that the reduced physiological form of selenium, hydrogen selenide, could be included as the fourth gasotransmitter, and exhibit non-canonical biological activity. This work assesses the current known biological actions of selenium-derivatives, its potential for non-canonical biological activity, how hydrogen selenide fits within the characteristic requirements of gasotransmitters, and the therapeutic potential of novel selenomimetics.

Does nitric oxide (NO) potentiate the anti-tumoral activity of pepper fruit extracts?



José M. Palma[1], José Pérez del Palacio[2], Marta Rodríguez-Ruiz[1], Salvador González-Gordo[1], Caridad Díaz[2], Carmen Ramos[2], Francisca Vicente[2], Francisco J. Corpas[1]

[1]Group of Antioxidant, Free Radicals and Nitric Oxide in Biotechnology, Food and Agriculture, Estación Experimental del Zaidín, CSIC, 18008 Granada, Spain; [2]Dpt. Screening & Target Validation, Fundación MEDINA, 18016 Granada, Spain;

Introduction: Plants (crops, medicinal, aromatic, etc.) are precursors of a wide variety of secondary metabolites that can also be used by humans for consumption and health benefit. Pepper (*Capsicum annuum* L.) fruit is characterized by its high amounts of antioxidants (vitamins C and A), polyphenols and capsaicinoids, among others. Particular attention has been paid to capsaicin, which is exclusive from pepper fruits, whose anti-inflammatory, antiproliferative and analgesic activities have been proven. Very recently, an important role for nitric oxide (NO) in the physiology and the metabolism of pepper fruits has been reported. **Aims:** Due to the potential interest of pepper metabolites for human consumption, the identification of new bioactive compounds of this crop and their modulation by NO gas was carried out. **Experimental design:** Fresh crude extracts from Sweet pepper fruits were prepared, and then, an untargeted metabolomic approach, using liquid chromatography coupled to mass spectrometry (LC-MS) was followed. Raw data were filtered and then subjected to statistical analysis in order to identify main discriminant metabolites between specimens. The antiproliferative activity of those pepper extracts against several tumoral cell lines was achieved. **Results:** Twelve main bioactive compounds were identified in sweet pepper fruits, including quercetin and its derivatives, L-tryptophan, phytosphingosin, FAD, gingerglycolipid A, tetrahydropentoxylin, blumenol C glucoside, colnelenic acid and capsoside A. The abundance of these metabolites varied depending on the ripening stage of the fruits, either immature green or ripe red, but it was also modulated upon treatment with exogenous NO gas. The metabolic pattern followed by quercetin and its derivatives, as a consequence of the ripening stage and NO treatment, was corroborated by transcriptomic analysis of genes involved in the synthesis of these compounds. Preliminary assays showed that crude extracts from pepper fruits displayed antiproliferative activity against HEPG2 (hepatoma) and MIA Paca-2 (pancreas) cells. **Conclusions:** This research opens new windows on the

pepper fruit's bioactive compounds with nutraceutical and biomedical potentiality. Considering the effect of ripening and NO, field practices and biotechnological strategies can be envisaged as tools to optimize the level of these beneficial compounds.

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Nitric oxide-releasing gemcitabine: a new weapon against pancreatic cancer?

Francesca Masetto[1], Konstantin Chegaev[2], Elena Gazzano[3], Nidula Mullappilly[1], Barbara Rolando[2], Silvia Arpicco[2], Roberta Fruttero[2], Massimo Donadelli[1], Chiara Riganti[3]

[1]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy [2]Department of Drug Science and Technology, University of Turin, Italy. [3]Department of Oncology, University of Turin, Italy

Objectives/Hypothesis: Nitric-oxide (NO) is a non-competitive inhibitor of drug efflux ATP binding cassette (ABC) transporters such as P-glycoprotein and multidrug resistance related proteins (MRPs) [1,2]. NO-conjugated doxorubicins or their liposomal formulations are effective against ABC transporter-expressing tumors, where the free drug fails [3,4]. We hypothesized that NO-conjugated gemcitabine (GEM) could be exploited in overcoming the chemoresistance mediated by ABC transporters in pancreatic ductal adenocarcinoma (PDAC). **Introduction.** PDAC is a therapy recalcitrant disease characterized by aberrations in multiple genes that drive pathogenesis and drug chemoresistance. GEM is the first-line treatment, but it is poorly effective. One cause of this failure is the expression of the drug efflux transporter MRP5 that extrudes GEM. **Aims:** We aim to find a pharmacological strategy alternative to GEM, able to overcome the resistance mediated by MRP5. **Experimental:** We synthesized a library of seven novel NO-releasing gemcitabine prodrugs (NO-GEMs), to improve the efficacy of GEM by exploiting the therapeutic effects of NO. Among these prodrugs we selected **5b** as the most effective compound to be tested in PANC-01 and Mia-Paca-2, two PDAC cell lines with different resistance to GEM and different levels of MRP5. To improve intratumor delivery, solubility and stability, we encapsulated the lead compound in liposomes producing the formulation **Lipo 5b**. **Results:** NO donors as diethylamine NONOate increased the cytotoxicity of GEM in MRP5-expressing PANC-01 cells, more resistant to GEM than the poorly MRP5-expressing Mia-Paca-2 cells. In keeping with this result, the lead NO-

releasing GEM **5b** dramatically reduced cell proliferation in PANC-01 cells, but it showed mild advantages over GEM in Mia-Paca-2 cells. **Lipo 5b** was a stable formulation, and it was internalized by PDAC cells in a time- and dose-dependent manner. Compared to free GEM and **5b, Lipo5b** increased the intracellular NO level, induced stronger cell cycle arrest and apoptosis by up-regulating Bim protein, caused a stronger DNA damage. These effects were NO-dependent since they were reversed by the NO scavenger PTIO. Notably, in a panel of PDAC cells with different resistance to GEM, sensitive cells had lower levels of MRP5, while resistant cells had higher levels of MRP5. **Lipo5b** nitrated MRP5 and reduced the efflux of GEM. This mechanism could explain the efficacy of **Lipo5b** in GEM-resistant PDAC cells, over-expressing MRP5. The key role of this transporter was demonstrated in MRP5 silenced cells, where the NO-releasing pro-drug lost its superior efficacy compared to GEM. **Conclusions**: Our results support the development of a new anti-tumoral strategy based on NO-GEM prodrugs, able to reverse the resistance to GEM in PDAC cells.

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YOUNG INVESTIGATOR SESSION

Unraveling the role and regulation of S-nitrosoglutathione reductase in breast cancer progression

Valeria Fiorentini[1], Salvatore Rizza[1,2], Fiorella Faienza[1], Paola Giglio[1], Gennaro Pepe[1], Giuseppe Filomeni[1,2]

[1]Department of Biology, University of Rome Tor Vergata, Rome, Italy; [2]Redox Biology Group, Danish Cancer Research Center, Copenhagen, Denmark

S-nitrosylation (SNO), the major post-translational modification induced by nitric oxide (NO), regulates several cellular processes implicated in the onset and progression of human disease, such as cancer. Protein-SNOs removal is regulated by a denitrosylase called *S*-nitrosoglutathione reductase (GSNOR). GSNOR decrease has been associated with cancer onset and progression, a feature suggesting it can act as a tumor suppressor. *In silico* analyses of gene

profiling, indicate that GSNOR is hypo-expressed in breast cancer (BC) and this correlates with worse prognosis. How GSNOR is downregulated in tumors and how it can influence tumor progression is still understood. To achieve this goal, we over and downregulate GSNOR expression in different subtype of cancer cell line (e.g., MCF7, MDA-MB-231) and we evaluate how this can influence cell proliferation, migration, and invasion. Moreover, we know that gene encoding GSNOR (*ADH5*) can be regulated epigenetically by specific microRNAs (miRNAs). Trough *in silico* analysis, we have identified miRNAs up regulated in BC and at the same time targeting GSNOR. We will try to inhibit this epigenetics effectors responsible for decreased GSNOR expression in BC to evaluate the effects on tumor phenotype.

Defining the role of GSNOR in BC cell proliferation, migration and invasion could shed new light on the current knowledge about the molecular mechanisms underlying BC progression and help provide new therapeutically relevant targets for BC treatments.

TRAP1 regulation by NO and ROS highlight its potential role as a mitochondrial redox sensor

Fiorella Faienza[1], Salvatore Rizza[2], Chiara Pecorari[2], Valeria Fiorentini[1], Paola Giglio[1], Claudio Laquatra[3], Carlos Sanchez-Martin[3], Giovanni Chiappetta[4], Francesca Pacello[1], Andrea Battistoni[1], Elena Papaleo[5], Andrea Rasola[3], Giuseppe Filomeni[1,2,6]

[1]Department of Biology, University of Rome Tor Vergata, Rome, Italy. [2]Redox Biology Group, Danish Cancer Society Research Center, Copenhagen, Denmark. [3]Department of Biomedical Sciences, University of Padova, Padova, Italy. [4]Laboratory of Proteomics and Biological Mass Spectrometry, USR, CNRS - ESPCI Paris, Paris, France. [5]Computational Biology Laboratory, Danish Cancer Society Research Center, Copenhagen, Denmark. [6]Center for Healthy Aging, University of Copenhagen, Denmark.

Objectives/Hypothesis. The mitochondrial chaperone TRAP1 senses nitrosative/oxidative stress via cysteine modifications and changes its activity and structure to maintain mitochondrial homeostasis. **Introduction.** TRAP1 (Tumor Necrosis Factor Receptor-associated Protein 1) is the mitochondrial paralog of Hsp90 family of molecular chaperones and is considered a protooncogenic molecule. It has a pivotal role in the maintenance of mitochondrial homeostasis, due to its ability to regulate OXPHOS and mitochondrial permeability transition pore (mPTP) opening (Masgras et al.,

2017). Moreover, it exerts protective functions by controlling the mitochondrial generation of ROS (Guzzo et al., 2014), although it is still not clear if and how TRAP1 senses ROS levels inside mitochondria. Our group demonstrated that TRAP1 is target of S-nitrosylation at Cys501. This makes TRAP1 prone to be degraded via the proteasome (Rizza et al., 2016) and affects its ATPase activity, modulating cell death response to staurosporine treatment (Faienza et al., 2020). Others reported that TRAP1 cys residues can also undergo sulfenylation (Yang et al., 2014; Akter et al., 2018), opening the possibility that TRAP1 adapts cell response by integrating different redox environmental information. **Aims.** The present work aims at understanding the impact of different redox modifications on TRAP1 structure, activity and functions with relevance to cancer. **Experimental design.** As *in vitro* model, we used TRAP1 recombinant proteins (WT or cysteine redox mutants) exposed to nitric oxide (NO) or hydrogen peroxide (H2O2) to induce redox modifications. As *in vivo* models we used tumor cell lines (expressing WT or cysteine redox mutants of TRAP1 subjected to oxidative or nitrosative stress in tumoral cellular models to observe: i) changes in protein structure by redox western blot; ii) changes in cellular responses by the identification of TRAP1 interactors and the characterization of pathways involved. **Results.** Redox SDS-PAGE and Western blot analyses of recombinant TRAP1 variants revealed the formation of redox-sensitive oligomers. All cysteine residues seem to be involved in TRAP1 oligomerization, with Cys261 and Cys573 having a prominent role, likely by the formation of inter-molecular disulfide bridges. Interestingly, SILAC mass spectrometry identified Keap1 as a significantly changing interactor of TRAP1 upon H2O2 exposure, suggesting that Nrf2-Keap1 axis might be modulated by the redox-modified TRAP1. **Conclusions.** In this study, we provide evidence that different oxidizing conditions produce TRAP1 oligomers, whose formation relies on the engagement of inter-molecular disulfide bridges involving mainly Cys261 and Cys573, However, we cannot rule out that other cysteines are also involved, with a relevance that depends on the stimulus applied. Overall, our results suggest that TRAP1 could act as “redox antenna” that activates different responses to NO and ROS through the oxidation of different sets of cysteines (e.g., the selective activation of Keap1- Nrf2 axis by ROS, which might argue for the anti-oxidant response being induced). However, how NO and ROS-induced modifications impact on TRAP1 oligomerization – and how this translates into different TRAP1 functions and cell responses – is currently under investigation.

The induction of peroxynitrite generation by Sorafenib plays a relevant role during mitochondrial dysfunction in liver cancer cells

Elena Navarro-Villarán[1,2,3], Patricia de la Cruz-Ojeda[1,2,3], Jordi Muntané[1,2,3]

[1]Institute of Biomedicine of Seville (IBiS), Hospital University "Virgen del Rocío"/CSIC/University of Seville, Spain. [2]Biomedical Research Center for Hepatic and Digestive Diseases (CIBERehd), Madrid, Spain. [3]Department of Medical Physiology and Biophysics, University of Seville, Seville, Spain.

Objectives/Hypothesis: The induction of oxidative and nitrosative stress plays a role during mitochondrial dysfunction induced by Sorafenib in liver cancer cells. **Introduction:** Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and the fourth most frequent cause of cancer-related death worldwide. Sorafenib is the first line recommended therapy for patients with locally advanced/metastatic HCC. The proapoptotic and antiproliferative properties of Sorafenib are associated with mitochondrial dysfunction associated with superoxide anion ($O_2^{\cdot -}$) and nitric oxide (NO) generations, and drastic reduction of cell respiration and ATP synthesis in HepG2 cells. The inhibition of NO by L-NAME recovered mitochondrial respiration in Sorafenib-treated HepG2 cells. **Specific Aims**: The aim of the study was the identification of the molecular mechanism by which $O_2^{\cdot -}$ and NO impact mitochondrial dysfunction in Sorafenib-treated HepG2. **Experimental design:** Sorafenib (0-10 µM) was administered to HepG2 cell line. The reduction of reactive oxygen or nitrogen species was assessed using: MnTBAP (superoxide dismutase or SOD mimetic 100 µM), FeTPPS (peroxynitrite or $ONOO^{\cdot}$ decomposition, 50 µM) and catalase (500 U/ml). Taking into consideration the previously shown activity of MnTBAP on $ONOO^{\cdot}$ and $O_2^{\cdot -}$ decomposition, and the 100-1000-fold less constant rate of MnTBAP versus SOD, it feasible that MnTBAP also targets $ONOO^{\cdot}$. Different parameters related to mitochondrial respiration and glycolytic rate (Seahorse), ATP, $O_2^{\cdot -}$ (Mitosox), NO (nitrite+nitrate and DAF2), UCP2 expression and its tyrosine nitration were determined. **Results:** Sorafenib (10 µM) dramatically reduced mitochondrial respiration, glycolytic rates and ATP generation, while increased mitochondrial hyperpolarization, $O_2^{\cdot -}$ and NO generation as well as UCP2 tyrosine nitration in Sorafenib-treated HepG2. These effects appeared to be counterbalanced by the administration of MnTBAP and FeTPPS. In particular, the treatments reduced UCP2 tyrosine nitration and $O_2^{\cdot -}$ generation, recovered basal and maximal mitochondrial respiration, and ATP levels in Sorafenib-treated HepG2 cells. FeTPPS moderately upregulated glycolysis in control and Sorafenib-treated cells. Interestingly, the administration of catalase induced a shift from glycolytic to oxidative respiration in Sorafenib-treated cells. **Conclusions:** The induction of mitochondrial dysfunction induced by Sorafenib was related to $ONOO^{\cdot}$ generation and UCP2 tyrosine nitration in HepG2 cells. The administration of MnTBAP and FeTPPS reduced UCP2 tyrosine nitration, $O_2^{\cdot -}$ generation and recovered mitochondrial respiration and ATP synthesis in Sorafenib-treated HepG2 cells.

Investigating the role of NO in cancer stem cell pathophysiology

Giasemi Eptaminitaki[1], Benjamin Bonavida[2], Stavroula Baritaki[1]

[1] Laboratory of Experimental Oncology, Division of Surgery, Medical School, University of Crete, Heraklion 71003, Crete, Greece; [2]Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, USA.

Nitric oxide (NO) is an intriguing free radical that has resurfaced in the last 15 years after much debate as to its pathogenicity in cancer. NO regulates critical aspects of cancer induction and progression including cell proliferation, apoptosis, metastasis and chemo/immune-resistance, while it can modulate the immunosuppressive tumor microenvironment and angiogenesis. The most striking feature of NO is that it can exhibit a dose, time and compartment-dependency, so that at high concentrations it acts as the source of nitrosative and oxidative stress, causing DNA damage and mitochondrial dysfunction along with upregulating apoptosis, while at low concentrations it decreases apoptosis and promotes angiogenesis, thus displaying tumoricidal roles. As such, NO has been gaining popularity in the designing of novel anticancer approaches based on the modulation of the intra- and extra-cellular NO levels mediated by NO donors and inhibitors. Recent findings have further confirmed the contribution of NO metabolism in defining the 'stemness' properties of cancer stem cells (CSCs) through cross-regulation of several cellular signaling pathways, including the Notch and Wnt cascades. The pluripotent and renewing properties of CSCs allow the initial tumor seeding events to take place and eventual propagation and metastasis, which are responsible for the bulk of failures of many conventional therapies and poor cancer survival rates.

Here we overview the literature on the impact of NO on CSC functions and properties evaluated mainly in the context of the cellular redox status. Given that most of the main CSC markers, such as Oct4 are redox sensitive factors, it has been suggested that the levels of intratumoral NO may be dictated as prognostic indicators of the oxidative stress-dependent 'stemness maintenance" or differentiation processes, that are selectively taking place in CSCs especially during infection-related carcinogenesis. As such, a cellular redox status sustained by low ROX and NO levels, maintains stem cell self-renewal properties, while under increased oxidative stress mediated by high ROS and NO concentrations the cellular redox status alters in favor of the stem cell differentiation. Accumulating evidence further suggests that low (but above basal/pathologic) levels of NO produced by eNOS, the NOS isoform linked with the lowest NO production among the three isoforms, inhibits apoptosis and promotes angiogenesis, tumor cell proliferation, mobility and invasiveness that are all characteristics of CSCs. Concomitant with this notion are our recent findings

showing that CD133+/CD44+ CSCs isolated from pancreatic adenocarcinoma (PDAC) have lower expression of constitutive eNOs mRNA, compared to non-stem cell like CD133-/CD44- phenotype. eNOs reduction in CD133+/CD44+ CSCs may be attributed to the elevated expression of its pro-inflammatory cytokine suppressors, IFN-γ, TNF-a and IL-1β, which also accompany the CSC phenotype. Similarly to eNOs, cGMP levels were also found decreased in PDAC CSCs, thus demonstrating deregulation of the NO/sGC/cGMP signaling pathway that may lead to reduced CSC differentiation rates. Accordingly, our preliminary findings on YY1 and Snail oncogene overexpression in CD44+/CD133+/CD24+ prostate CSCs and the previously shown transcriptional induction of Prostate Stem Cell Antigen (PSCA) by YY1, as well as the DETANONoate-mediated YY1 and Snail S-nitrosylation and inactivation, may suggest putative NO targets that maintain prostate cancer cell "stemness".

Summarizing, there is a large body of evidence that points to NO impact on cancer stem cell behavior, as it relates to the maintenance of a stem-like or differentiated cell phenotype. Nevertheless, further research is necessary to shed more light into the mechanisms by which different NO levels may affect CSC pathophysiology.