BenBedPhar Training School

NRF2 in Noncommunicable Diseases: from Bench to Bedside

PROGRAM AND ABSTRACTS

Smolenice Castle, Slovakia
June 26—30, 2023

cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

MINISTRY
OF EDUCATION, SCIENCE,
RESEARCH AND SPORT
OF THE SLOVAK REPUBLIC

CENTRE OF EXPERIMENTAL MEDICINE
SLOVAK ACADEMY OF SCIENCES

WELCOME

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Dear BenBedPhar colleagues,

One of the most relevant COST tools is the implementation of training schools. Three training schools will be organized by BebBedPhar as part of our "capacity building objectives". The end goal is to promote a timely scientific community of NRF2 basic, pharmacological, and clinical researchers and entrepreneurs and to develop a "sense of belonging" to the EU scientific community. Our first training school is possible thanks to the positive and enthusiastic temper of Dr. Iveta Bernatova and the local organizers in Slovakia. They will make this training school a very successful vehicle for multidisciplinary training and international interaction of trainers and trainees.

Antonio Cuadrado

Chair of COST Action CA20121, BenBedPhar

cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

WELCOME

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Dear trainers, dear trainees,

It is my great pleasure to welcome you to the Training School of the COST CA20121 ***"Bench to Bedside Transition for Pharmacological Regulation of NRF2 in Noncommunicable Diseases (BenBedPhar)",*** *which takes place in the Congress Centre of the Slovak Academy of Sciences at Smolenice Castle in Slovakia. The Training School aims to provide comprehensive knowledge on the transcription factor NRF2 function which is a master regulator of multiple cytoprotective responses and a key molecular link among various noncommunicable diseases.*

During the Training School, distinguished scientists, the experts in NRF2 research, will present you with state-of-the-art knowledge on the role of NRF2 during aging, under stress, and in diseased states. They will also present the possibilities of pharmacological modulation of NRF2 function, tools for studying NRF2 as well as new perspectives of treatment of NRF2 associated disorders.

In addition, you will have the opportunity to present the results of your research. Discussions with experts and informal discussions of all participants will be an important part of the Training School that can help you accelerate your career growth.

Last but not least, an important benefit of this action is gaining new contacts, start networking and establishing personal friendships, which can significantly influence your further scientific interests.

I believe that the picturesque premises of the Congress Centre of Smolenice Castle and its surroundings will contribute to a good working atmosphere and positive mood during your stay.

On behalf of the local organizers, I wish you a pleasant stay in Smolenice Castle.

Iveta Bernatova

LOCAL ORGANIZERS

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Iveta Bernátová, head of the Local Organizing Committee (Action Management Committee member), senior researcher, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

Peter Bališ, researcher, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

Michal Kluknavský, postdoc, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

Andrea Mičurová, PhD. Student, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

VENUE

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Smolenice Castle

Congress Centre of the Slovak Academy of Sciences, Zamocka 18, Smolenice, Slovakia

Information

kcsmolenice.sav.sk/en/

CONTENTS

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

CONTENTS

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

PROGRAM OVERVIEW

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Central European Time	Monday June 26, 2023	Tuesday June 27, 2023	Wednesday June 28, 2023	Thursday June 29, 2023	Friday June 30, 2023
8:00-9:00	14:00 Departure from Bratislava to Smolenice Castle	Breakfast	Breakfast	Breakfast	Breakfast and check-out
9:00 -09:25		**NRF2 in inflammation** A. Dinkova-Kostova	**NRF2 and cardio-vascular diseases** A. Grochot-Przeczek	**NRF2 in liver diseases** A. Cuadrado	
9:25-9:50				**Implication of NRF2 in depression** M.G. Lopez	**NRF2 pharmacology** A. Dinkova-Kostova
9:50 -10:15		**NRF2 in ageing** I. Trougakos	**NRF2 in neuro-degenerative diseases** M. G. Lopez	**NRF2 in cancer and radiotherapy** G. Manda	**Tools to study NRF2** A. Grochot-Przeczek
10:15-10:40				**NRF2 in non-mammalian species** I. Trougakos	10:15-10:45 **2 Trainees' oral presentations**
10:40-11:15		Coffee break	Coffee break	Coffee break	Coffee break
11:15-12:05		**NRF2 in stress responses** I. Bernatova	**6 Trainees' oral presentations**	**Poster viewing and discussions**	11:15-12:15 **4 Trainees' oral presentations**
12:05 -12:55		**NRF2 biomarkers in blood** G. Manda			12: 15 **Concluding remarks** A. Cuadrado
12:55 -14:00		Lunch	Lunch	Lunch	Lunch
14:00-16:00		**Poster viewing and discussions**	**Social program: Hiking, Cave Driny visit**	**Social program: Excursion to Red Stone Castle**	**14:00 Departure from Smolenice Castle to Bratislava**
16:00-16:30	**Registration and accommodation**	Coffee break			
16:30-17:00		Meet the Experts: Students–Teachers Discussion AC, GM			
17:00-17:30	**Opening and General introduc-tion to NRF2** A. Cuadrado	Meet the Experts: Students–Teachers Discussion ADK, IT			
17:30-18:00		Meet the Experts: Students –Teachers Discussion AGP, MGL, IB			
18:00-18:30	**Social program Smolenice Castle Tour**		**Practical presentations** I. Bernatova, J. Markus		
18:30-19:00		18:15 Student mixer			
19:00-19:30	Break	Break	Break	Break	
19:30-21:30	**Welcome dinner**	**Dinner & music**	**Dinner**	**Farewell BBQ**	

DETAILED PROGRAM

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Monday, June 26, 2023

16:00 – 17:00 **Registration and accommodation**

17:00 – 17:10 **Opening**

17:10 – 18:00 **General introduction to NRF2.** A. Cuadrado; Spain,

18:00 – 19:00 Smolenice Castle Tour

19:30 – 22:00 Welcome Dinner

Tuesday, June 27, 2023

09:00 – 09:50 **NRF2 in inflammation.** A. Dinkova-Kostova

09:50 – 10:40 **NRF2 in aging.** I. Trougakos

10:40 – 11:15 Coffee Break

11:15 – 12:05 **NRF2 in stress responses.** I. Bernatova

12:05 – 12:55 **NRF2 biomarkers in blood**. G. Manda

12:55 – 14:00 Lunch

14:00 – 16:00 **Poster session I**

16:00 – 16:30 Coffee Break

16:30 – 17:00 **Meet the Experts A. Cuadrado and G. Manda** (Students-teachers discussion)

17:00 – 17:30 **Meet the Experts A. Dinkova-Kostova and I. Trougakos** (Students-teachers discussion)

17:30 – 18:15 **Meet the Experts A. Grochot-Przeczek, M. G. López and I. Bernatova** (Students-teachers discussion)

18:15 – 19:00 **Student Mixer** (Student discussion)

19:30 – 22:00 Dinner & Music

DETAILED PROGRAM

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Wednesday, June 28, 2023

09:00 – 09:50 **NRF2 and cardiovascular diseases.** A. Grochot-Przeczek

09:50 – 10:40 **NRF2 in neuro-degenerative diseases.** M. G. Lopez

10:40 – 11:15 Coffee Break

Trainees' oral presentations

Chairpersons: D. Carnicero-Senabre, I. Oblak

11:15 – 11:30 **Ivana Kuntic, GERMANY,** Activation of NRF2 by environmental stressors provides an opportunity for fast screening reporter assays for evaluation of biological toxicity

11:30 – 11:45 **Eduardo Cazalla Ibanez, SPAIN,** Impact of NRF2-BACH1 Signaling on Angpt1/2-TIE2 pathway: Implications for Blood-Brain Barrier Integrity

11:45 – 12:00 **Shara N. Sosa Cabrera, AUSTRIA,** AMP(K)lifying Nrf2 : Role of AMPK activity for Nrf2-mediated cytoprotection in a Keap1 -/- background

12:00 – 12:15 **Lucia, Viqueira Diaz-Alejo, SPAIN,** Microglial repopulation: short-term, long-term, and enforced with an Nrf2 inducer in the context of tau pathology

12:15 – 12:30 **Yang Luo, NEDERLAND,** Thiosulfate sulfurtransferase deficiency promotes cerebral cortical oxidative distress resulting in antioxidant system dysfunction and aberrant NRF2 function

12:30 – 12:45 **Ivan, Lucic, CROATIA,** The role of AQP3 in the NRF2/KEAP1 and EGFR/PI3K/AKT signaling pathways in breast cancer cell lines

12:55 – 14:00 Lunch

14:00 – 18:00 Social program, Cave Driny visit (hiking)

18:00 – 19:00 **Practical presentations.** I. Bernatova, J. Markus

DETAILED PROGRAM

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Thursday, June 29, 2023

09:00 – 09:25 **NRF2 in liver diseases.** A. Cuadrado

09:25 – 09:50 **Implication of NRF2 in depression.** M. G. Lopez

09:50 – 10:15 **NRF2 in cancer and radiotherapy**. G. Manda

09:50 – 10:40 **NRF2 in non-mammalian species.** I. Trougakos

10:40 – 11:15 Coffee Break

11:15 – 12:55 **Poster session II**

12:55 – 14:00 Lunch

14:00 – 19:00 Social program

19:30 – 22:00 Farewell BBQ

Friday, June 30, 2023

09:25 – 09:50 **NRF2 pharmacology.** A. Dinkova-Kostova

09:50 – 10:15 **Tools to study NRF2.** A. Grochot-Przeczek

Trainees' oral presentations

Chairpersons: S.N. Sosa Cabrera, A. Micurova

10:15 – 10:30 **Francesca Prestia, ITALY,** Deciphering the interplay between the unfolded protein response and insulin resistance in Down syndrome neuropathology

10:30 – 10:45 **Arif K. Salihoglu, TURKIYE** , NRF2 downregulation and neuroendocrine modifications of intergenerational hippocampal effects in gestational diabetes mellitus

10:45 – 11:15 Coffee Break

11:15 – 11:30 **Maria J. Caballero Herrero**, **SPAIN**, Role of ND-13, a DJ-1/Nrf2 pathway activator, in the prevention of inflammasome activation in diabetic nephropathy

11:30 – 11:45 **Georgios Psarias, SWITZERLAND,** Screening of natural compounds for antioxidant transcriptional activity and effects on the thyroid

11:45 – 12:00 **Margarida Pedro, PORTUGAL,** Development of a screening pipeline to identify modulators of NRF2 activity

12:00 – 12:15 **Miroslav Novak, UK,** Pharmacological NRF2 activation: electrophiles vs PPI inhibitors

12:15 – 12:45 **Concluding remarks**

12:55 – 14:00 Lunch

14:00 – Departure to Bratislava

POSTER SESSION I

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Tuesday, June 27, 2023

Chairpersons: G. Psarias, F. Prestia

1. **Viktorija Maksimova, NORTH MACEDONIA,** Piperine as a natural derived NRF2 stimulator in prevention or therapy of ROS induced diseases

2. **Jose Jimenez-Villegas, SPAIN**, Exploring the intersection of NRF2, stress granule dynamics, and RNA metabolism in ALS

3. **Muhammet Kamaran, TURKIYE**, Disabling of Nrf2 activation via inhibition of NADPH metabolism-related antioxidant enzymes

4. **Andrea Micurova, SLOVAKIA**, Different role of NRF2 in the liver and heart of rats exposed to polyethylene glycol-coated magnetite nanoparticles

5. **Marina Oskomic, CROATIA**, Protein interactions of DPP3 and their putative impact on NRF2-KEAP1 signaling

6. **Patricia Pavelkova, SLOVAKIA**, The comparison of molecular hydrogen administration methods on radiation-induced heart damage

7. **Thomas Dixon, UK**, Evaluation of the mechanisms by which activation of the transcription factor Nrf2 inhibits lung fibrosis

8. **Lucrezia Romana Rolfi, ITALY**, Trisomy21 and aberrant BACH1/Nrf-2 axis: implications for neurodegeneration

POSTER SESSION II

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Thursday, June 29, 2023

Chairpersons: A. K. Salihoglu, V. Maksimova

9. **Valentina S. Rumanova, SLOVAKIA,** Effects of dim light at night on daily rhythm in Nrf2 expression and metabolism in rats

10. **Iza Oblak**, **SLOVENIA**, NRF2 and drug-induced liver injury

11. **Adriana Martiskova, SLOVAKIA**, Role of oxidative stress in heart failure

12. **Daniel Carnicero-Senabre, SPAIN,** The role of NRF2 in synaptic homeostasis

13. **Hatice Esenkaya, TURKIYE**, Investigating the effects of G-quadruplex structures in NRF2

14. **Mercedes Vallejo Mudarra, SPAIN,** Nrf2 in acute kidney injury associated with rhabdomyolysis

15. **Michal Kluknavský, SLOVAKIA**, ACE2 Inhibitor MLN-4760 elevates expression of *Nfe2l2* and antioxidant genes in the brainstem of spontaneously hypertensive rats

16. **Livia Gajdosova, SLOVAKIA,** The effect of a combined intervention (omega-3 fatty acids and physical activity) on functional parameters of sarcopenia and oxidative stress in aged rats

17. **Louisa Watt, UK.**

TRAINERS' LECTURES

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

ANTONIO CUADRADO

Department of Biochemistry, Medical College, Autonomous University of Madrid, Madrid, Spain. E-mail: antonio.cuadrado@uam.es

Prof. Antonio Cuadrado is a full professor of Biochemistry and Molecular Biology at the Department of Biochemistry, Medical School, Autonomous University of Madrid. He obtained his PhD degree in Biology in 1985 and enjoyed several postdoctoral stays in the National Cancer Institute -NIH with the help of Fulbright and Fogarty fellowships. He established his independent laboratory as Professor of Biochemistry in 1997 with a main interest on the study of molecular mechanisms involved in initiation and progression of chronic diseases. For the past years his main lane of research has been the validation of transcription factor NRF2, master regulator of cellular homeostasis as a new therapeutic target in chronic diseases with particular emphasis in neurodegenerative diseases (Alzheimer and Parkinson) and in fatty liver diseases. His current interest is the development of new NRF2-modulating drugs. Dr. Cuadrado has published over 160 primary and review articles, of which more than 80 are related to the role of NRF2 in physiological and pathological responses to disease.

Presentation I: General introduction to NRF2

Transcription factor Nrf2 (Nuclear factor (erythroid-derived 2)-like 2) is a master regulator of cellular homeostasis that controls the expression of more than 1% of human genes related to biotransformation reactions, redox homeostasis, energy metabolism, DNA repair, and proteostasis. These genes possess a cis-acting regulatory sequence termed antioxidant response element (ARE). Structurally, it is a member of the cap 'n' collar (CNC) subfamily of basic region leucine zipper (bZip) transcription factors and makes heterodimers with other bZip proteins, of which small MAFs G, K and F are the best characterized. Its activity has a tremendous impact on physiology and pathology and therefore it is very tightly regulated by a complex array of transcriptional regulators and post-

translational modifications, that ensure proper transcriptional activity under basal conditions and under adaptation to environmental changes. The main mechanism of regulation of NRF2 is at the level of protein stability. Most studies have focused on the role of the electrophile and redox sensor Kelch-like ECH-associated protein 1 (KEAP1) to adjust NRF2 protein levels to metabolic demands. KEAP1 interacts with two regions of NRF2 (amino-acid sequences DLG and ETGE) located at the Neh2 N-terminal domain to direct ubiquitination by the Cullin-3/Rbx1 complex and proteasome degradation of NRF2. On electrophilic modification or oxidation of KEAP1, the interaction with NRF2 is disrupted. Then, NRF2 escapes degradation and targets ARE genes to increase the capacity of antioxidant and biotransformation reactions. In addition to the very well established regulation by the ubiquitin E3 ligase adapter KEAP1, another mechanism of NRF2 regulation is based on signaling pathways that regulate glycogen synthase kinse-3(GSK-3).This kinase phosphorylates specific serine residues in the Neh6 domain of NRF2 to create a degradation domain that is then recognized by the ubiquitin ligase adapter β-TrCP and tagged for proteasome degradation by a Cullin1/Rbx1 complex. Several electrophilic compounds induce NRF2 due to sulfhydryl modification of specific redox sensitive cysteines in redox sensor proteins such as KEAP1 and PTEN and thus impact on NRF2 stability by either of these two mechanisms. Many of these compounds have been used as nutraceuticals and some of them have reached clinical evidence. The most successful case so far reported is the ester derivative of fumaric acid, dimethyl fumarate (DMF). DMF crosses the gastrointestinal barrier where it is converted into monomethyl fumarate (MMF). Some very potent synthetic triterpenoids have been studied in teh context of diabetic nephropathy (bardoxolone methyl) or Friedreich ataxia (omaveloxone). Another NRF2 inducer that has reached the level of clinical studies is the isothiocyanate sulforaphane (SFN) isolated from broccoli sprout extracts. Considering that Nrf2 elicits a defense against multiple stress conditions, it has shown its negative side in some concers where somatic mutations lead to it constitutive activations. New strategies are being developed to inhibit NRF2 under these conditions. We will discuss along this course the mechanistic regulation of NRF2 and its impact in physiology and pathology.

Presentation II: NRF2 in liver diseases

Around 23-25% of adults worldwide have Non-alcoholic fatty liver disease (NAFLD) and among them 20% develop non-alcoholic steatohepatitis (NASH), which, together with its comorbidities, is one of the main causes of mortality. At this time, there is not a drug specifically approved for the treatment of NASH, which probably reflects that NASH is a clinical manifestation that gathers several pathomechanisms. In fact, NASH is characterized by the presence of oxidative stress, inflammation, and metabolic alterations. These three crucial hallmarks can be targeted with a single hit by activating the transcription factor Nuclear factor erythroid 2 Related Factor 2 (NRF2). In this lecture, we will review the mechanisms that point NRF2 as a promising target to endorse a disease modifying therapy for NASH and fibrosis. Three E3 ubiquitin ligases appear to control NRF2 protein levels in liver: Kelch-like ECH-associated protein 1 (KEAP1), β-transducin repeat-containing protein (β-TrCP), and HMG-CoA reductase degradation protein 1 (Hrd1, also called synoviolin (SYVN1)). Many processes that downregulate NRF2 are triggered by transforming growth factor-beta (TGF-β), with oxidative stress amplifying its signaling. In animal models, knockout of NRF2 increases susceptibility to NASH, while pharmacological activation of NRF2 by inducing agents that target KEAP1 or beta-TrCP prevent NASH development, or if NASH has been initiated, suppresses liver steatosis and progression towards fibrosis. There is therefore compelling evidence that pharmacological activation of NRF2 may be a comprehensive strategy to treat NASH and fibrosis.

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

GINA MANDA

Radiobiology Laboratory, Victor Babes National Institute of Pathology, Bucharest, Romania, Email: gina.manda@ivb.ro

Prof. Gina Manda is a senior scientist, Head of the Radiobiology Department at "Victor Babeș" National Institute of Pathology, Bucharest, Romania. She has an H-index of 20 with 148 ISI-indexed papers and more than 1800 citations. She is the Vice-leader of the COST Action CA20121 BenBedPhar. She studied Physics at the University of Bucharest (1985) and holds a Ph.D. in Biophysics (1996). Her actual research is focused on redox biology and immunology in pathology (neurodegeneration), radiobiology (radiotoxicology) and cancer therapy (new radiotherapeutic approaches and photodynamic therapy). Through collaboration with the European Space Agency, she is testing NRF2 therapeutics for protection against the deleterious effects of galactic cosmic rays on astronauts. In addition, she is a member of the BIOSPHERE consortium focused on the synergic cellular action of terrestrial secondary cosmic rays and UV radiation.

Presentation I: NRF2 biomarkers in blood

Low-grade oxidative stress and inflammation seem to precede the onset of overt symptoms in most of chronic noncommunicable diseases due to the deregulation the NRF2 signaling pathway at local and systemic level. Recent clinical evidence of NRF2 disturbance in chronic diseases will be summarized. The relevance of NRF2 as cellular or soluble biomarker in blood will be discussed as a non-invasive approach for monitoring disease since its early stages or NRF2-targeted therapeutic strategies addressing both redox disturbances and inflammation. Transcriptomic data on the NRF2 molecular fingerprint in the blood of patients with Alzheimer's disease versus age-matched controls will be discussed.

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

Presentation II: NRF2 in cancer and radiotherapy

The aberrant chronic activation of the NRF2 signaling pathway in various types of tumor cells, including cancer stem cells, has been shown to be associated with poor prognosis. Mutations in the KEAP1-NRF2 system or at the level of ARE, deregulated epigenetic control and redox signaling account for the constitutive NRF2 activation which provides to tumor cells a survival advantage, and makes them highly chemo- and radioresistant. Accordingly, adjuvant down-regulation of the NRF2 system is expected to sensitize tumor cells to therapy, but NRF2 inhibitors are still far from being translated from bench to bedside. In turn, NRF2 can protect normal cells against early stages of carcinogenesis, making its pharmacologic activation relevant for patients at risk to develop cancer or which are exposed to carcinogens. Transcriptomic data on the NRF2 signature in normal and tumor cells exposed to radio- or photodynamic therapies will be discussed.

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

ALBENA T. DINKOVA-KOSTOVA

Division of Cellular and Systems Medicine, University of Dundee School of Medicine, United Kingdom, Email: a.dinkovakostova@dundee.ac.uk

Prof. Albena T. Dinkova-Kostova is a Professor of Chemical Biology at the University of Dundee School of Medicine (UK). She graduated in Biochemistry and Microbiology from Sofia University (Bulgaria) and obtained her PhD degree in Biochemistry and Biophysics from Washington State University (USA). She subsequently trained in Pharmacology at Johns Hopkins University School of Medicine (USA), where she continues to hold an Adjunct Professor position. She joined the University of Dundee in 2007 as a Research Councils UK Academic Fellow and a research group leader. Her group collaborates with basic scientists and clinicians, and with the pharmaceutical industry. In her research, at the interface of Chemical Biology and Medicine, she is committed to understanding how cells and organisms respond to oxidative, inflammatory, and metabolic stress, and is working towards development of strategies for protection against chronic disease. She was named among the top influential academics in Clarivate's Highly Cited Researchers 2019, 2020, 2021 and 2022 lists.

Presentation I: NRF2 in inflammation

The transcription factor nuclear factor erythroid 2 p45-related factor 2 (NRF2; encoded by *NFE2L2*) and its principal negative regulator, Kelch-like ECH associated protein 1 (KEAP1), control the expression of large networks of genes encoding cytoprotective proteins that provide adaptation to oxidative, electrophilic, inflammatory, and metabolic stress. The role of NRF2 in drug metabolism has been known since the discovery of the transcription factor in the 1990s. A decade later, a structure-activity study of a large series of synthetic triterpenoid analogues of oleanolic acid, which were originally developed as inhibitors of cellular inflammatory processes, revealed a linear correlation spanning 6 orders of magnitude of concentration between the anti-inflammatory andcompounds representing seven chemically distinct classes of NRF2 activators identified VNRF2-activating activities of these compounds. A follow-up study of structurally-diverse compounds representing seven chemically distinct classes of NRF2 activators

identified suppression of inflammation as a consistent property of NRF2 activators. This correlation suggested that the two activities of these compounds are mechanistically linked. Subsequent experiments in NRF2-deficient murine macrophages demonstrated that the anti-inflammatory activity of such compounds is partly dependent on NRF2. In addition to xenobiotics, NRF2 is also activated by endogenous metabolites, such as the cis-aconitate-derived itaconate, which accumulates to millimolar concentrations in inflammatory macrophages, and has a crucial role for the resolution of inflammation. Most recently, high-resolution quantitative proteomics showed that NRF2 is a critical factor governing redox and intermediary metabolism and facilitating mitochondrial adaptation in macrophages encountering pro-inflammatory stimuli. This presentation will provide an overview of the anti-inflammatory role of NRF2 activation in a number of experimental systems, including primary murine macrophages, animal models, and peripheral blood mononuclear cells (PBMCs) and skin of human subjects.

Presentation II: NRF2 pharmacology

Inducible transcription factor nuclear factor erythroid 2 p45-related factor 2 (NRF2; encoded by *NFE2L2*) is a member of the human cap'n'collar (CNC) basic-region leucine zipper transcription factor family. The protein products of its target genes perform versatile cytoprotective functions, including antioxidant, anti-inflammatory, metabolic and drug-metabolizing, and have roles in the maintenance of protein homeostasis. Through its transcriptional targets, NRF2 activation orchestrates a comprehensive and long-lasting protection that allows adaptation and survival under diverse forms of cellular and organismal stress. Pharmacologic NRF2 activators have shown protective effects in numerous models of human disease and benefits in human intervention trials, and NRF2 is an attractive therapeutic target, with several NRF2 activators in various stages of drug development, and one compound, dimethyl fumarate, in clinical practice for the treatment of remitting-relapsing multiple sclerosis and psoriasis. The advances in drug development of NRF2 modulators are however accompanied by numerous challenges, including target specificity, monitoring target engagement/pharmacodynamic responses, short/long-term safety considerations, identifying the most appropriate disease indications, and understanding the extent and implications of variation in NRF2 activity. This presentation will outline the rationale for targeting NRF2, and the recent advances and challenges in drug development of NRF2 activators.

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

ANNA GROCHOT-PRZĘCZEK

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland, Email: anna.grochot-przeczek@uj.edu.pl

Anna Grochot-Przeczek is an associate professor in the Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics, and Biotechnology, Jagiellonian University in Krakow, Poland. She studies the molecular mechanisms that regulate the function of endothelial cells and blood vessels with a focus on NRF2/KEAP1 pathway, ageing and S-nitrosation. Currently, she investigates the importance of NRF2/KEAP1 imbalance and loss of proteostasis in the function of blood vessels.

Presentation I: NRF2 and cardiovascular diseases

Cardiovascular diseases (CVDs), a group of disorders of the heart and blood vessels, are the leading cause of death worldwide. Many of them are associated with impairment of the defence mechanisms against oxidative stress. Therefore, NRF2 being a master orchestrator of cellular stress response is an interesting subject of cardiovascular research. In the talk, I will give an overview of the mechanisms related to the role of NRF2 in cardiovascular diseases, such as atherosclerosis, heart failure, and abdominal aortic aneurysm.

Presentation II: Tools to study NRF2

Using the right tools is a key for a precise understanding of the molecular mechanisms that determine physiological and pathological conditions. In the field of NRF2 we have many tools which help us investigate its importance in various experimental settings. However, it is critical to know several subtle details that may affect data interpretation. In the talk, I will present several misconceptions that linger in the literature.

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

MANUELA G. LOPEZ

Department of Pharmacology, Medical School and Institute Teofilo Hernando. Autonomous University of Madrid, Spain, Email: manuela.garcia@uam.es

Prof. Manuela G. Lopez is MD PhD and full professor of Pharmacology at the Department of Pharmacology in the School of Medicine, Universidad Autónoma de Madrid (UAM), Spain. Currently, she heads the Institute Teofilo Hernando for drug discovery (http://www.ifth.es/) that belongs to UAM. Her group, "NeuroprotectionLab" (http://neurodiscovery-ndd.com/gt1), has particular interest in the identification of new potential therapeutic targets to develop innovative and disease modifying therapies for neurodegenerative diseases, with special focus in modulating neuroinflammation (microglia-astrocyte interaction), oxidative stress and autophagy. Within the field of NRF2, she has contributed to the understanding of NRF2 in pain, depression, stroke and neurodegenerative diseases, together with the development of different NRF2 multitarget drugs in collaboration with medicinal chemists. Currently, she is coordinating a drug development project to identify Keap1-NRF2 inhibitors with potential use in Alzheimer's disease (AD).

Presentation I: NRF2 in neurodegenerative diseases

In this presentation we will go through the evidence from preclinical to clinical studies that indicate that NRF2 is dysfunctional in different neurodegenerative diseases. Studies from NRF2 knockout mice have been very helpful to determine how this transcription factor can impact in Alzheimer´s disease pathology. Once shown the proof of concept that induction of NRF2 could be an innovative strategy to develop drugs to achieve a disease modifying effect for AD and other related dementias, we will present the student an example to understand the drug development process for an NRF2 inducer for AD.

Presentation II: Implication of NRF2 in Depression

In this lecture, we will review the major hypotheses on the physiopathology of depression. Current treatments for depression are based mainly on the monoaminergic or serotoninergic dysfunction hypotheses; however, the response rates of these drugs are limited (around 50 % after 4 weeks treatment). Focusing in the more recent inflammatory hypothesis and data from NRF2 knockout animals, we will envision that NRF2 induction may be an interesting alternative to develop new drugs with a totally novel mechanism of action for depression. We will present preclinical data that will support this hypothesis, including the effects on an endogenous NRF2 compound-agmatine. Finally, we will present evidence obtained from patients with major depression.

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

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Ioannis Trougakos obtained his Ph.D. in Cellular-Developmental Biology from the National and Kapodistrian University of Athens (NKUA), Greece. He has worked as Research Scientist at EMBL, Germany, CBM "Severo Ochoa", Spain and at NHRF, Athens, Greece; he was also research visitor at EMBL and at the Netherlands Cancer Institute. Dr. Trougakos was elected Research Lecturer at NHRF and currently he serves as Professor and Director of the "Cell Biology" lab at the Faculty of Biology, NKUA. Dr. Trougakos has published articles (>190) in high-ranking journals, chapters in international books and he co-authored an academic book (citations ~19500; h-Index = 45 / i10-index = 129); he is also co-inventor in several patents. His group is funded by private (GR, EU, USA) and public (GR, EU) entities; also, the group participates in contractual activities with the Industry.

Presentation I: NRF2 in ageing

Aging is a complex phenomenon caused by the time-dependent loss of cellular homeodynamics and consequently of physiological organismal functions. This process is affected by both genetic and environmental (e.g., diet) factors, as well as by their constant interaction. The balanced functionality of (among others) cellular antioxidant and proteostatic modules is central to genome, proteome and mitochondrial stability. The antioxidant response system comprising (among others) the ubiquitously expressed NFE2 -related transcription factor 2 (NRF2) and its redox-sensitive cytoplasmic inhibitor Kelch-like ECH-associated protein 1 (KEAP1) defends tissues against oxidative stress, thereby protecting against pathologies that relate to DNA, protein, and/or lipid oxidative damage. These NRF2 functions, along with the extensive functional wiring of genomic,

proteostatic and mitostatic modules, become less efficient in higher metazoans during advanced age. The gradual dysfunction of the NRF2/KEAP1 regulatory network during aging is (among others) a driving force for most age-related diseases.

Presentation II: NRF2 in non-mammalian species

A central module of antioxidant defenses in higher metazoans refers to the ubiquitously expressed NFE2-related transcription factor 2 (NRF2), which along with its redox-sensitive cytoplasmic inhibitor Kelch-like ECH-associated protein 1 (KEAP1), defends tissues against unbalanced oxidative load, providing thus protection against oxidative damage of biomolecules. The NRF2-KEAP1 system is seemingly evolutionarily conserved in different organisms of the animal kingdom and studies in lower animals and model organisms (e.g., zebrafish, *Drosophila melanogaster* and *Caenorhabditis elegans*), including recent advances in genome projects, have provided important information regarding the evolvement of these anti-stress machineries during evolution. We will discuss the NRF2-KEAP1 system in non-mammalian model animals, providing also input on the evolutionary history of this ancient cell defense system.

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

IVETA BERNATOVA

Department of Experimental Hypertension, Institute of Normal and Pathological Physiology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia

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Iveta Bernatova is a senior scientist, Head of the Department of Experimental Hypertension Centre of Experimental Medicine, v.v.i., Slovak Academy of Sciences, Bratislava, Slovakia. She studied Biochemistry, holds a Ph.D. degree in Chemistry and scientific degree Doctor of Science (D.Sc., an equivalent of research professor) in Animal Physiology. She completed postdoctoral stay at the School of Medicine, Wright State University, Dayton, Ohio in Physiology and Pharmacology. Her research is focused on integrative physiology, mainly on the mechanisms of blood pressure regulation in various experimental models. Currently she is focused on the role of NRF2 in chronic social stress-induced alteration in the heart and livers with focus on iron metabolism.

Presentation I: NRF2 in stress responses

Chronic stress is considered a risk factor associated with the development of various non-communicable diseases. Activation of stress systems leads to a cascade of neuroendocrine, cardiovascular, behavioral, metabolic and immune responses to ensure the integrity and survival of the organism. Yet, long-lasting and/or intensive stressors can lead to diseased states, in which oxidative stress and inflammation are present. NRF2, among others, is involved in the regulation of various metabolic pathways such as glucose metabolism, fatty acid synthesis, iron and heme metabolism as well as glutathione synthesis and utilization. This lecture will focus on systemic and molecular mechanisms in stress that are regulated by NRF2.

Practical presentation

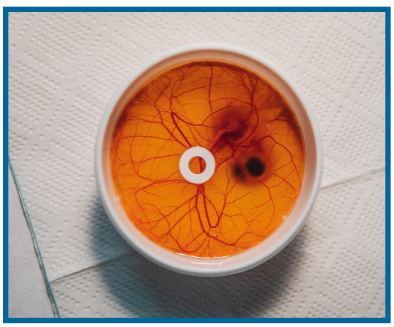
The aim of this session is to show the participants selected alternative laboratory methods that are in line with the 3R (Replacement, Reduction and Refinement) principle in animal experimentation. Participants will see the alternative methods of testing the effects of various substances without the use of laboratory animals.

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

MATTEK
A BICO COMPANY

Use of in vitro models of human epithelia in toxicology, pharmacology and medical research will be presented by **Dr. Jan Markus**, production manager and senior scientist from MatTek, Slovakia. Animal models have been widely applied for assessing bioavailability and toxicity of drugs and other substances. However, such testing has many pitfalls, including ethical issues and often limited translatability to human conditions. Hence, in vitro models are needed to guide the design of molecules or dosing schedules that mitigate safety risks in humans. Besides that, these models are successfully used to predict substance toxicity as well as to model infectious and non-infectious diseases and study therapeutical modalities. Over the years we have generated and successfully implemented 3D reconstructed models of many types of human epithelia. In this brief practical demonstration we will discuss selected established and new models and demonstrate their use in the various biological testing scenarios. Hands-on practice will be available to interested participants.

Iveta Bernatova introduces you to the chicken chorioallantoic membrane method. It is a relatively simple in vivo method, suitable for modelling more complex systems, as an intermediate step between in vitro and mammalian models. The advantage of CAM is the rapid growth of blood vessels, the availability of vessels of various diameters and the complete availability of the circulatory system (for intravascular administration of substances). The method allows visualization of tests in real time, good reproducibility and reliability of testing. It is a cost-effective method compared to animal models, allowing multiple tests to be performed on individual CAMs.



TRAINEES' ABSTRACTS

Ivana Kuntić

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Position: PhD. Student

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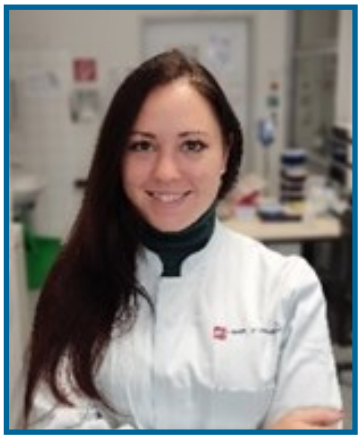

Ivana Kuntić studied Pharmacy at the University Business Academy in Novi Sad (finished masters in 2020), and is currently a PhD student in the group of prof. Andreas Daiber at the University Medical Center Mainz, Germany. She is a member of several national and international scientific communities (SfRBM, SFRRE, ASBMB, DGK), and a holder of SfRBM Trainee Award 2021. Her PhD studies are oriented toward environmental and behavioural risk factors (e.g. noise, shisha smoke, particulate matter), which generated two published research articles and one manuscript that is currently prepared for submission that were presented at several international conferences. Special research interests: redox biochemistry, oxidative stress and environmental research in cardiovascular disease.

Activation of NRF2 by environmental stressors provides an opportunity for fast screening reporter assays for evaluation of biological toxicity

Ivana Kuntić, Marin Kuntic, Hartmut Kleinert, Andreas Daiber

Air pollution, noise, ultraviolet radiation, chemical agents and mental stress are some of the known environmental risk factors that have significant impact on human health. Molecular pathomechanisms connected to the environmental risk factors are often mediated by oxidative stress and inflammation. Nuclear factor erythroid 2-related factor 2 (NRF2) is a transcription factor that is known to be activated by oxidative stress and inflammation. One of the ways to show NRF2 activation is by determining the levels of heme oxygenase 1 (HO-1) protein, a target of NRF2 regulated gene transcription. Here, we discuss the potential use of the luciferase-linked HO-1 promoter activity assay (HO-1-prom) as a read-out of NRF2 activation, using DLD-1-HO-1-prom reporter cells. We tested different sizes of particulate matter (PM), ranging from nanometer- to micrometer-size carbon black particles, as well as ambient PM (NIST particles SRM 1648a), in order to probe the impact of air pollution-derived PM on NRF2 activation. As a proof that PM exposure induces HO-1 expression by NRF2 activation, we tested specific NRF2 inhibitors (ML385 and AEM1). To investigate whether PM-derived oxidative stress activates the NRF2-pathway, we also incubated cells with direct reactive oxygen species (ROS) scavengers (TEMPO, PEG-SOD and PEG-catalase). Mitochondrial ROS formation was tested using mitochondria-targeted ROS scavenger mitoTEMPO. To differentiate between direct PM-derived ROS and indirect cellular ROS formation by PM, we tested NADPH-oxidase inhibitors GSK2795039 and VAS2870. Cell viability was used as a marker of biological toxicity in correlation with HO-1 induction. Other environmental toxins and risk factors will also be tested in future studies.

Eduardo Cazalla Ibáñez

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Position: PhD. Student

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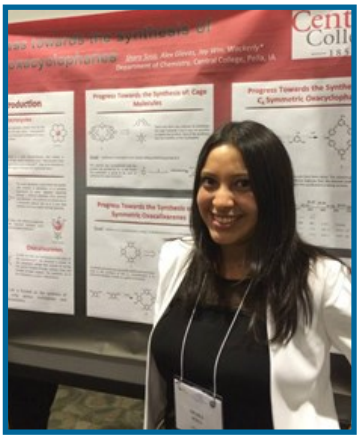
Eduardo Cazalla completed his Biology degree at the Autonomous University of Madrid in 2021. He is currently embarking on his PhD studies, supervised by Dr. Antonio Cuadrado and Dr. Ángel Juan García-Yagüe. Eduardo's research project is focused on the connection between NRF2 and BBB stability in an Alzheimer's disease context, focusing on the ANGPT1/2-TIE2 pathway.

Impact of NRF2-BACH1 Signaling on Angpt1/2-TIE2 pathway: Implications for Blood-Brain Barrier Integrity

Background: The selective permeability of molecules from the bloodstream to the brain is governed by the Blood-Brain Barrier (BBB), which relies on the ANGPT1/2-TIE2 signalling pathway to maintain its properties. The integrity of the BBB is compromised under neuropathological conditions, where endothelial cells are subjected to oxidative and inflammatory stress. To protect against such stress, NRF2 activates a genetic program, which may play a role in preserving the BBB. However, the relationship between the ANGPT1/2-TIE2 pathway and NRF2 has not been thoroughly investigated. Our study examined the impact of chemical and genetic activation of NRF2 on *TEK/Tek* expression, which encodes TIE2, following an in-silico search resulting in AREs regions located in the gene sequence.
Methods: In this study, we assessed changes in the NRF2 pathway and TIE2 expression levels using Western blotting and qRT-PCR. We also evaluated changes in TIE2 levels through immunofluorescence, along with cell junction proteins. Overexpression and silencing of NRF2 were accomplished through non-replicative lentiviral vectors.
Results: The activation of NRF2 by isothiocyanates or overexpression led to a decrease in TIE2 levels in brain endothelial cells. In contrast, the transcription factor BACH1, a classical repressor of NRF2 activity, had the opposite effect, promoting *TEK/Tek* regulation. We compared the effects of the NRF2/TIE2 axis on an inflammation model induced by lipopolysaccharide (LPS), which also caused a decrease in TIE2 levels. Furthermore, besides TIE2 downregulation, which could implicate a loss of integrity, NRF2 was seen to increase the expression of multiple tight junction proteins correlating with a more stable barrier.
Conclusions: While most ARE genes are activated by NRF2 and repressed by BACH1, our findings suggest that *TEK/Tek* belongs to a small number of ARE genes that exhibit the opposite regulation. Nevertheless, NRF2 presents other protective roles in maintaining BBB as seen by changes in cell junction protein levels.



Shara Natalia Sosa Cabrera

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I graduated from the Chemistry master's program at the University of Vienna. The title of my thesis project is "Effect of 5-CQA on Nrf2 activity in non-tumorigenic and tumorigenic human colon cells". Therefore, pursuing a Ph.D. in the same field was the next obvious step in my career I got interested and enthusiastic to continue working on Nrf2. That is why I apply, and got, for a PhD candidate position at Professor Dr. Elke Heiss in the pharmaceutical sciences department. The main goal of my Ph.D. project is to provide an unprecedented and deeper understanding of the interaction between AMPK and the transcription factor Nrf2. This basic knowledge could be useful to design future rational approaches to optimally boost or weaken cellular stress resilience in situations of increasing age (when cellular defences decline, and redox/metabolic imbalance rise) or cancer (where cellular stress resistance impedes therapeutic success).

Publications: (https://doi.org/10.1016/j.freeradbiomed.2022.07.014).

AMP(K)lifying Nrf2: Role of AMPK activity for Nrf2-mediated cytoprotection in a Keap1$^{-/-}$ background

Life is continuously exposing our cells to stressful insults. In order to adapt /restore homeostasis and prevent damage, several mechanisms evolved that fight and counteract these stressors and lend cells resilience. One player in the context of pending oxidative and xenobiotic harm is the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) (Osama et al., 2020a) which is a product of the NFE2L2 gene (Fouzder et al., 2021a; Lovatt et al., 2020). In the context of metabolic/energy stress, AMP-activated protein (AMPK) enters the stage as main sensor of cellular energy status in all eukaryotic cells (Garcia and Shaw, 2017a) and central regulator of energy homeostasis. The methods used up to date are several cell culture techniques, RT qPCR, confocal microscopy, western blot, IP and CoIP, plasmid amplification an isolation, transfection. So far, we tried to answer the following questions: Q1: Can AMPK activators alter levels of Nrf2, Nrf2 target genes or Bach1 in lung cancer cells? NO Q2: Can AMPK activation alter sensitivity to oxaliplatin? NO Q3: How can AMPK mediate any observed effect on the molecular level? Is the effect Nrf2 dependent or rather due to another binder of Keap (which might be unleashed from Keap control in the Keap-/- background): currently working on the signal transduction pathway of metformin in A549 cells.

Lucía Viqueira Díaz-Alejo

Affiliation: Universidad Autónoma de Madrid, Madrid, Spain

Position: Predoctoral student

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I did my BSc in Biotechnology and I specialized in Animal and Human Biotechnology. During this period, I had the opportunity to spend one academic year at Rolniczy University in Krakow through an Erasmus+ scholarship. Subsequently, I completed the MSc in Pharmacological Research with my thesis entitled "Pharmacological evaluation of the multitarget NRF2 inducer-melatonin derivative as a potential drug for AD". Currently I am working on my Doctoral Thesis entitled "Microglia-astrocyte interaction in the progression of tauopathy: in search of new targets" with Ministry of Universities funding (FPU). **Research interests:** In order to understand the relationship between dysfunctional molecular processes and disease, I have always felt particularly drawn to Molecular Biology and the clinical uses of biotechnology. Moreover, neurodegenerative diseases are of great interest to me, particularly Alzheimer's Disease. Thus, Pharmacology as a field allows me to apply Molecular Biology basic knowledge for drug discovery in the context of disease prevention and treatment.

Microglial repopulation: short-term, long-term, and enforced with an Nrf2 inducer in the context of tau pathology.

Background: Recent GWAS studies have demonstrated the presence of LOAD risk variants in proteins that are mainly expressed in microglia, highlighting the importance of these cells in the onset and progression of AD. Microglial survival and proliferation highly depends on the signalling via CSF1R and can be depleted by the administration of CSF1R inhibitors such as PLX5622. **Methods:** Stereotaxic AAV-hTau injection was performed to C57BL/6J mice and PLX5622 was used to deplete microglia. Then, the CSF1R inhibitor was removed, and microglia was repopulated in a short (21 days) and long-term (56 days) basis; in this last case in the presence or absence of an Nrf2 inducer. Different behavioural tests and immunofluorescence studies were conducted. **Results:** Short-term microglial repopulation is protective in an induced tauopathy model, by reversing neuronal loss, cognitive decline assessed by NOR and OLT, and restoring to similar control levels the nº of Iba1+ cells, CD68 intensity in Iba+ cells and microglial activation. Nevertheless, when the repopulation period was extended protection was lost. Finally, we performed an enforced repopulation in the presence of an Nrf2 inducer which was able to reverse this cognitive loss. **Conclusions:** Short-term microglial repopulation prevents cognitive decline in the context of tau pathology. In the long-run, this benefitial effect is lost, whereas in the presence of an Nrf2, neuroprotection is mantained over time.

Yang Luo

Affiliation: University of Groningen (RUG)&University Medical Center Groningen (UMCG), Groningen, Netherlands

Position: PhD candidate

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Education: Sep 2014 – Jun 2018 Bachelor, Nankai University,CN, BSc (Hons) Pharmaceutical technology, Sep 2018 – Sep 2019 Postgraduate, King's College London, UK, MSc Pharmaceutical Analysis and Quality Control, Distinction, March 2021- Now Ph.D. candidate, RUG & UMCG. NL.
Jan 2022- Dec 2022 EIT Ageing School PhD Program
Research interest: The key role of thiosulfate sulfurtransferase in ferroptotic pathway and oxidative stress in the brain

Thiosulfate sulfurtransferase deficiency promotes cerebral cortical oxidative distress resulting in antioxidant system sysfunction and aberrant NRF2 function

Background: Besides amyloid and tau buildup, neurodegeneration is also associated with oxidative distress. Thiosulfate sulfurtransferase(TST, EC 2.8.1.1) was discovered as a mitochondrial rhodanese and beneficial characteristics relate to antioxidant defense has been identified. However, the importance of TST during oxidative distress remains obscure.

Methods: WT and $Tst^{-/-}$ C57BL/6J mice brains were collected, and antioxidant enzymes protein and activity levels via immunoblot and specific kits. The OXPHOS proteins and respiratory activities were measured by immunoblot and Oroboros. Fluorescent probes were utilized to measure ROS and RSS. Total ATP level was detected by luminescence. Nrf2 signaling pathway was detected via qRT-PCR. Glutamate content is measured via commercial kit.

Results: $Tst^{-/-}$ mice are characterized by 20-fold and 475-fold elevation in the trunk plasma and urine of thiosulfate compared to WT mice. The $Tst^{-/-}$ mice cortex exhibits significantly decrease H_2S and significantly increased polysulfides (H_2S_n) levels, but a dysregulated pathway downsteam of H_2S as evidenced by a significantly decreased GSH/GSSG ratio. We also observed significant total ATP level increase in the $Tst^{-/-}$ cortexes, which correlated with no significant difference in OXPHOS complex proteins, and a significant Complex IV respiratory capacity increase. These data indicate increase in mitochondrial fitness. In addition, H_2O_2 and O^{2-}, including as products of OXPHOS, showed a significant increase in $Tst^{-/-}$ cortical brain samples. Finally, we found a significant decrease in SOD activity in $Tst^{-/-}$ cortexes compared with WT. The catalase activity in $Tst^{-/-}$ was 10% higher compared to WT. And a significant Nrf2 signaling pathway activation failure.

Conclusions: Overall, our study unrevealed that TST deficiency promoted dysregulation of the reactive species interactome through both ROS and RSS polysulfides overgeneration coupled with mitochondrial OXPHOS remodeling. TST deficiency elicits the dysregulation of GSH antioxidant and Nrf2-keap1 pathway activation.

Ivan Lučić

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Position: PhD. Student

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I am Ivan Lučić, a PhD student working in the Laboratory for Oxidative Stress, Division of Molecular Medicine at Ruđer Bošković Institute. Although my background is limited as I have just started working, I have been actively involved in several research projects within my laboratory. My master's thesis focused on the interaction between aquaporin 3 and the EGFR/PI3K/AKT signaling pathway in breast cancer cell lines. For my PhD research, I will further investigate the relationship between breast cancer, oxidative stress, and NRF2.

The role of AQP3 in the NRF2/KEAP1 and EGFR/PI3K/AKT signaling pathways in breast cancer cell lines

Background: Aquaporin 3 (AQP3), a membrane pore, is overexpressed in numerous tumors, including breast tumors. AQP3 promotes cell migration and invasion. PI3K/AKT and NRF2 pathways promote breast cancer growth and therapy resistance and are interconnected. Recent research suggests a connection between AQP3 and PI3K/AKT signaling, while there is no evidence of whether AQP3 and NRF2 interconnect. The aim of this work was to investigate whether and how AQP3 affects NRF2/KEAP1 and EGFR/PI3K/AKT signaling pathways upon EGF stimuli and/or lipid raft disruption in breast cancer cell lines.

Methods: Three representative breast cancer cell lines were used: MCF7 (hormone receptors-positive), SkBr-3 (HER2 receptor-positive), and SUM 159 (triple-negative). Basal and AQP3-silenced cells were treated with MBCD (to disrupt lipid rafts) and/or EGF. Cell viability was measured, and protein expression of NRF2, KEAP1, PI3K, AKT, p-AKT, and AQP3 were investigated. The ratio of p-AKT/AKT was used to assess the activation of the PI3K/AKT signaling pathway.

Results: In MCF7 cells, EGF stimuli and/or lipid raft disruption increased cell viability and activated PI3K/AKT signaling pathway regardless of AQP3-silencing. An increase in NRF2 was observed in unsilenced MCF7 cells upon EGF treatment. EGF stimuli affected SkBr-3 cells, leading to increased viability following lipid raft disruption and activation of the PI3K/AKT signaling pathway in AQP3-silenced cells. Both treatments increased the expression of NRF2 in SkBr-3 cells. Lipid raft disruption followed by EGF stimuli decreased SUM 159 viability regardless of AQP3 presence, while AQP3-silencing decreased cell response to EGF stimuli. The decrease in viability was accompanied by PI3K/AKT signaling pathway activation and an increase in NRF2 expression.

Conclusions: The results indicate that the response of the breast cancer cell lines to EGF stimuli and/or lipid raft disruption differed, and the expression of NRF2 and KEAP1 was affected by AQP3, suggesting a potential link between them that requires further investigation.

Francesca Prestia

Affiliation: La Sapienza, University of Rome, Rome, Italy

Position: PhD. Student

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Pharmaceutical chemistry and Technology graduate at the University of Calabria in 2020. Completed a MSc's studentship carrying out a research project in Polymers and Biomaterials of pharmaceutical interest, entitled 'Theranostic Nanosystems for drug targeting of sunitinib maleate'. Passionate about academic research, completed a post-graduate internship "Demetra" project aimed to perform toxicological characterization of potentially contaminated substance. Started a PhD program in Biochemistry in 2021 at the University of Rome, focusing my study on analyzing molecular mechanism of Alzheimer-like neuropathology in Down Syndrome associated with loss of proteostasis.

Deciphering the interplay between the unfolded protein response and insulin resistance in Down syndrome neuropathology

Background: Protein homeostasis (proteostasis) is essential for normal brain function and the unfolded protein response (UPR) holds a key role in its preservation. A maladaptive response, such as chronic UPR activation, provides a link between the accumulation of misfolded proteins, increased oxidative damage and neurotoxicity. We recently reported that the dysregulation of the PERK branch of the UPR is associated with reduced Nrf2 antioxidant response contributing to the progression of Alzheimer-like signatures in Down syndrome (DS) brain. In addition, our studies support the notion that in DS brain insulin resistance (BIR) and mitochondrial defects advance in parallel to faulty proteostasis and are associated with redox imbalance and cognitive decline. Our study aimed to understand the pronicity of DS phenotype in developing defects of proteostasis under aberrant metabolic stimuli and to unravel the role of trisomic genes (such as BACH1) in exacerbating the toxic partnership between BIR and UPR. **Methods:** We administered primary neurons and astrocytes for WT mice, and lymphoblastoid cells (LCLs) isolated from DS patients with insulin and palmitic acid (IPA) to mime BIR and subsequently, we treated cells with UPR targeting agents. In parallel, we investigated the role of BIR and UPR in DS mice subjected to high fat diet (HFD). **Results:** Our data on primary cortical neurons and astrocytes demonstrated that IPA led to the de-regulation of the PERK/eIF2a axis, which resulted in the reduction of protein translation and Nrf2-related antioxidant responses. By pharmacologically targeting the UPR we were able to ameliorate metabolic defects associated to IPA treatment, improve proteostasis and reduce oxidative damage by the rescue of Nrf2 signalling. Similarly, the IPA-induced accumulation of tau and Aβ, observed in DS LCLs, is cleared after the administration of UPR targeting agents rescuing proteostasis and Nrf2 induction. Collected data on DS mice subjected to HFD reported reduced cognition associated with the development of BIR and the chronic deregulation of the PERK/Nrf2 axis. **Conclusions:** Our results suggest that metabolic defects occurring in DS (but also in normal population) exacerbate the failure to regulate the PERK pathway and is both cause and effect of Nrf2 signal depletion, representing an essential step in promoting aberrant proteostasis and neurodegeneration.

Arif Kamil Salihoğlu, MD, MRSB

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Position: PhD. candidate

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I am PhD student in the Department of Physiology, Karadeniz Technical University Faculty of Medicine. My research interests include autophagy/mitophagy dysmodulation and mitochondrial dysfunction in the pathogenesis of diabetic neuropathy, cell death mechanisms and neuroendocrine disturbances in neurodegenerative disorders; by methods in vivo, in vitro et in silico. I am an active member of many international physiological societies, and was accepted as a member of the Royal Society of Biology in July 2021. orcid.org/0000-0003-3864-9377, avesis.ktu.edu.tr/aks/publications.

NRF2 downregulation and neuroendocrine modifications of intergenerational hippocampal effects in gestational diabetes mellitus

Background: Intrauterine hyperglycemia is a prominent manifestation of gestational diabetes mellitus (GDM) and is related to a high risk of diabetes in new generations. However, the cellular mechanism of intergenerational hippocampal effects of GDM is not fully understood yet. The aim of this study was to detect this mechanism on examining differentially expressed genes (DEG) in a mouse model with intrauterine hyperglycemia exposure by using in silico tools.
Methods: GSE147039 dataset downloaded from Gene Expression Omnibus database was re-analysed for DEG profiles in the R program. (In the dataset, ICR mice at 16 weeks-old-age (n=5 control, n=5 first-generation -G1- GDM mice, n=5 second-generation -G2- GDM mice) were examined.) Based on Benjamini-Hochberg correction, adjusted p-values <0.05 were accepted as significant.
Results: DEG levels indicated that NRF2 expressions from G1 and G2 GDM mice (evaluated together) were down-regulated compared to the control ($p<0.037$, log of fold change "FC": 2.00) , and NRF2 expressions were up-regulated in G2 GDM group compared to G1 ($p=0.042$, logFC: 1.44). Meanwhile, NRF2 expressions were down-regulated in G2 GDM group, compared to control group ($p=0.017$, logFC: 1.28). Besides, neuroendocrine genes like preproenkephalin (PENK), pro-opiomelanocortin-alpha (POMC), oxytocin (OXT), glial cell line-derived neurotrophic factor (GDNF), diazepam binding inhibitor (DBI), angiotensinogen (AGT), tachykinin-1 (TAC1) genes were upregulated ($p<0.05$); and brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF1), neuropeptide-Y receptor (NPY2R), gastrin-releasing peptide (GRP), chromogranin B (CHGB) genes were downregulated ($p<0.05$) in GDM groups, compared with the control group.
Conclusions: Results from this study indicate NRF2 downregulation and imbalances in DEG levels (up- and down-regulation) of genes known to be involved in many neuroendocrine signalling in hippocampal cells during the developmental process of GDM.

María José Caballero Herrero

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"I am a biologist specialized in immunology who have participated as student evaluating the involvement of the tumor stroma in the "MHC class I and immune escape in colorectal cancer (CRC)" project funded by Carlos III Health Institute (Madrid, Spain). At the present, I am starting my career as PhD student under supervision of Dr. Santiago Cuevas. My main research project is focus on the study of the preventive effects of new Nrf2 pathway regulators drugs on the development of diabetic nephropathy, and their capacity to regulate inflammasome activation. In addition, I am collaborating on a project about CRC funded by Carlos III institute in the Morales Meseguer General University Hospital in the Region of Murcia, directed by the Dr. Graciela Navarro and co- directed by Dr. Santiago Cuevas. As a result of this collaboration, I have a co- first author publication on the field, tilled "Role of danger-associated molecular patterns (DAMPS) in the postoperative period after colorectal surgery" (Int J Mol Sci; Q1; DOI: 10.3390/ijms24043862).

Role of ND-13, a DJ-1/Nrf2 pathway activator, in the prevention of inflammasome activation in diabetic nephropathy

Background: Inflammasome is a crucial regulator of renal inflammation and a key factor in the pathogenesis of renal diseases. DJ-1 is a renal protein with antioxidant and anti-inflammatory properties with the capacity to prevent Nrf2 degradation. To explore new pharmacological applications of Nrf2/DJ-1 pathway we designed ND-13, a peptide consisting of 13 highly conserved amino acids from the DJ-1 sequence. **Methods:** Mouse bone marrow macrophages (BMM) were treated with Bardoxolone, a Nrf2 inducer, and ND-13. Diabetes was induced in C57Bl/6 mice via injection of streptozotocin (STZ) and treated with ND-13. PBMCs were isolated from patients with diabetic nephropathy and controls and were plate and stimulated with LPS/ATP and treated with ND-13 and MCC950. **Results:** The IL-1β concentration in the medium of BMM increased by NLRP3 inflammasome stimulation by LPS/ATP, ATP and decreased in macrophages pre-treated with Bardoxolone (65.07±26%, n=4, P<0.05) but not pre-treated with ND-13, however, in presence of H2O2 (100 nM), ND-13 significantly decreased IL-1β release after NLRP3 activation (88.6±1.2%, n=4, P<0.05). STZ treatment increased the IL-1β production compared with the control, suggesting that inflammasome may be activated in diabetes and ND- 13 treatment normalized its activity. PBMCs isolated from patients with diabetic nephropathy presented a trend to increase IL-1β release compared to controls and diabetic individuals and ND-13 could have a role in the prevention of inflammasome activation. **Conclusions:** All these data point out that DJ-1/Nrf2 pathway stimulation is a promising approach to decreasing immune cells inflammasome activation, and ND-13 could be a new approach to attenuate inflammation in renal diseases.

Georgios Psarias

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Georgios Psarias is an MPharm graduate (Dept. of Pharmacy University of Patras, Greece) who is currently studying in the position of Ph.D. at the University Hospital of Lausanne (CHUV). His main study Field is the function of the NFE2 family of transcription factors such as Nrf1 (NFE2-related factor 1) and Nrf2 and their role in regulating the cellular response to oxidative stress in the thyroid. Also, during his studies, He participated in global competitions organized by M.I.T regarding synthetic biology and its applications (iGEM). Before his Ph.D., he was a member of the Biology department of Hellenic Open University and worked for one year as a research intern on a project based on Epigenetics.

Screening of natural compounds for antioxidant transcriptional activity and effects on the thyroid

Background: Recent studies have identified pleiotropic roles for the Nrf2 antioxidant response in the physiology of the thyroid gland and in the pathophysiology of various thyroid diseases. Various natural compounds have antioxidant properties and/or thyroidal effects, but it is not well known whether and how the two are related. The aim of the present study was to characterize in a systematic manner the thyroidal and antioxidant effects of natural compounds. **Methods:** We performed a low-throughput manual chemical screen of >400 natural compounds in thyroid follicular cell lines stably transfected with reporter constructs. We used the rat PCCL3 cell line in its wild-type form as well as a PCCL3 Nrf2-knockout clone generated via CRISPR/Cas9 mutagenesis. The following read-outs were assessed: Nrf2 transcriptional activity (ARE-luciferase); Nrf1 protein stabilization (Nrf1-delta-luciferase); cell viability (CellTiter-Glo); reporter gene expression of the thyroid hormone precursor thyroglobulin (TG-luciferase); reporter gene expression of the sodium-iodide symporter (NIS-luciferase); and iodine uptake by the cells. For compounds showing activity in the respective assays, the mRNA and protein levels of NIS, TG and the antioxidant gene Nqo1 were assayed by qRT-PCR and Western blot, respectively. **Results:** Among other findings, we observed that certain compounds paradoxically induced higher transcriptional activation of the ARE in Nrf2-knockout cells than in wild-type cells. Further studies showed that these compounds were potent activators of Nrf1, which is highly expressed in the thyroid in vivo. As a further example, the flavonoid compound bavachin was found to increased iodine uptake by the cells in an Nrf2-independent manner. Importantly, bavachin was also able to reverse the decrease in iodine uptake that is induced by exposure to lithium, a drug used in the treatment of bipolar disorder. **Conclusions:** In conclusion, screening of natural compounds in thyroid cell lines can yield relevant hits with potential therapeutic relevance in thyroid diseases.

Margarida Pedro

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Young and motivated MSc student on Biochemisty for Health, with a background in Biology, currently developing ther master thesis entitled "*Development of a screening pipeline to identify modulators of NRF2 activity*". Recently accepted as a membre of the WG2 of bench to bedside transition for pharmacological regulation of NRF2 in non-communicable diseases (BenBedPhar)- CA20121.
Interested in molecular mechanisms of disease but looking foward for new oportunities.

Development of a screening pipeline to identify modulators of NRF2 activity

Background: Age-related macular degeneration (AMD) is the most common blinding disease in the western world and is currently incurable. The primary cause of its pathology is related to the retinal pigment epithelium (RPE) which presents NRF2 activity impairment in aged mice, leading to RPE damage and resembling AMD. The most known regulator of NRF2 is the E3 ligase adapter KEAP1, that drives it to its degradation by proteasome, under homeostatic conditions. However, under stress, KEAP1 is oxidized, leading to conformational changes of the NRF2/KEAP1 complex, preventing NRF2 degradation; newly synthesized NRF2 is translocated into the nucleus where it activates antioxidant, detoxification, anti-inflammatory, proteasome, and autophagy genes. Human phenolic metabolites (HPM) found in circulation after fruits and vegetables ingestion, are devoid of toxicity at circulating levels and cross the blood-brain barrier. Preliminary data point out that some HPMs can modulate NRF2 activity.
Methods: An *in silico* analysis based on molecular docking and molecular dynamics simulations between our pharmacological target, KEAP1, and the HPMs, is in progress. The more promising hits will be then tested in a stable RPE cell line containing an 8xARE-luc reporter, expressing luciferase when NRF2-ARE pathway is activated. We aim to validate the lead compounds in established cellular models of disease, such as the model of AMD in RPE cells.
Results: As a proof of concept, dimethyl fumarate (DMF), the only NRF2 activator approved by FDA and EMMA, is being used to optimize conditions and showed promising results activating the NRF2 pathway in RPE cells, as assessed by immunoblotting, RT-qPCR of target genes and immunofluorescence microscopy. Importantly, NRF2 activation by DMF shown to reduce AMD features in our model of AMD in RPE cells. Additionally, preliminary data with one of the HPM revealed promising results in activating NRF2 to even higher levels than DMF-driven activation. Promising results are therefore expected for the remaining HPM.
Conclusions: This study will contribute with new molecules capable of modulating NRF2 activity, that could possibly be used for the treatment of AMD patients.

Miroslav Novak

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After graduating from high school in Slovakia, I moved to Scotland, enrolling in the BSc Biological Sciences course at the University of Dundee. I chose advanced modules on signalling pathways, immunology, cancer biology, and genetics, before continuing to an MSci degree in immunology. Currently a third year PhD student in the lab of Professor Albena Dinkova-Kostova, I am experienced in cell culture, western blotting, qPCR, microscopy, flow cytometry. My interests lie mostly in the field of protein-protein interactions and the causation and causality of post-translational modifications.

Pharmacological NRF2 activation: electrophiles vs PPI inhibitors

Background: There are two types of pharmacological NRF2 activators: electrophiles and non-electrophilic protein-protein interaction (PPI) inhibitors. Both stabilise NRF2 by binding to KEAP1 and inhibiting KEAP1-dependent NRF2 degradation. Electrophiles react with cysteines in KEAP1; while KEAP1 is a redox-sensitive protein, reactive cysteines in other proteins can also be modified. As current PPI inhibitors are designed to bind the Kelch domain of KEAP1 specifically, the KEAP1-independent effects of electrophiles are expected to be avoided.
Methods: The potency of selected NRF2 activators was determined in human and murine cells using the NAD(P)H:quinone oxidoreductase (a classical NRF2 target) activity assay. Equivalent effective activator concentrations were used to confirm changes in the mRNA and protein levels of NRF2 and associated proteins by qPCR and western blotting during time-course experiments. The dependence of observed effects was tested by RNAi-mediated knockdown. Lastly, a cell line stably expressing KEAP1-mCherry was used for immunoprecipitation of KEAP1-bound proteins.
Results: PPI inhibitors show prolonged NRF2 stabilisation compared to electrophiles, and promote NRF2 phosphorylation. PPI inhibitors, but not electrophiles, increase KEAP1 levels, likely due to impaired p62-mediated KEAP1 turnover. Alongside NRF2, KEAP1 also binds p62 or PGAM5. Electrophiles increase the proportion of NRF2 among the proteins co-immunoprecipitating with KEAP1, whereas PPI inhibitors lead to a decrease in all co-immunoprecipitated proteins.
Conclusions: Electrophiles can affect multiple processes by modifying other proteins, in addition to KEAP1. Our findings suggest PPI inhibitors, although specific for KEAP1, may have other effects due to the displacement of non-NRF2 KEAP1 binders. This fact should be considered during KEAP1-targeting drug development.

Viktorija Maksimova

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Viktorija Maksimova is an associate professor in pharmaceutical botany and pharmacognosy at the Department of applied pharmacy, Faculty of Medical Sciences at the University of Goce Delcev in Shtip, North Macedonia. She is a Master of Pharmacy and defended her PhD thesis in the topic of Antioxidative and cytotoxic effect of capsicinoids, in 2016 at the same University. Currently, she's working on medicinal plants, and she is interested in researching small bioactive molecules as a novel superior antioxidant and cytotoxic agents among polyphenols and alkaloids. Recently she has become interested in studying plant bioactive molecules as NRF2 modulators.

Piperine as a natural derived NRF2 stimulator in prevention or therapy of ROS induced diseases

Background: Piperine is the major alkaloid represented in *Piper nigrum* (black pepper) showing different pharmacological properties that are still extensively studied. Piperine's ability to activate the protein expression levels of NRF-2 and HO-1 and inhibit the protein expression levels of Keap-1, is directly influencing the antioxidative capacity of the cells and ROS homeostasis.

Results: Activation of NRF2 by piperine has triggered an antioxidant response cell system (HO-1, GSH, CAT, SOD) scavenging ROS, and decreasing lipid peroxidation in colon cancer cells. These results indicate that piperine may be an effective molecule in prophylactic aims of colon carcinogenesis by targeting the NF-κB/NRF-2/Keap-1/HO-1 pathway. The novel effects of piperine in attenuating the oxidative stress in lung epithelial cells were shown recently. Treatment with piperine enhanced the NRF2 expression and reversed changes induced by cigarette smoke extract. Increased NRF2 levels promoted anti-inflammatory effect in the same cells. Piperine has shown protective effects against Aβ-induced neuronal damage and oxidative stress, in the SH-SY5Y cell model. Activation of NRF2 pathway can also lead to inhibition of LPS-induced inflammatory response in microglial cells. In addition, a novel piperine derivative, HJ105, obtained through structure-based design and optimization was revealed in 2021, as a potent small molecule for treatment of Alzheimer disease. This structure promoted effective suppression of Keap1-NRF2 complex formation, and additional neuroprotective mechanisms of HJ105 underlying apoptotic cell death, oxidative stress response and neuro-inflammation.

Conclusions: Piperine and even more its derivatives are attracting increasing attention for their anti-apoptotic, anti-inflammatory, anti-antioxidant, cytoprotective and cognitive enhancing effects.

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I hold a Bachelor's degree in Biochemistry and a Master's degree in Molecular Biomedicine from the Autonomous University of Madrid. Currently, I am a doctoral candidate in the Molecular Biosciences PhD program at the same university, analyzing the role of NRF2 in neurodegenerative diseases. My research interests include the intersection of redox biology, autophagy, and RNA metabolism in amyotrophic lateral sclerosis, combining tools from molecular biology and bioinformatic fields.

Exploring the intersection of NRF2, stress granule dynamics, and RNA metabolism in ALS

Background: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the death of motor neurons, for which no effective therapy is currently available. Several studies have linked ALS pathogenesis with dysregulation of RNA metabolism and redox homeostasis, suggesting a potential role for NRF2, a transcription factor involved in redox control, in the disease.
Methods: We performed a comprehensive analysis of transcriptomic differential expression studies comparing ALS patients and healthy individuals. We used a biological process network approach to identify dysregulated pathways consistently altered across studies. To investigate the molecular connection between NRF2 and RNA metabolism dysfunction in ALS, we searched for NRF2 binding motifs in genes related to this process found to be altered in ALS patients. We then evaluated the impact of NRF2 deficiency in RNA metabolism by monitoring stress granule dynamics, a critical process in RNA regulation, in response to sodium arsenite.
Results: Our analysis confirmed that RNA metabolism was significantly impaired in ALS patients. We identified 206 NRF2 binding motifs in genes related to RNA metabolism found to be dysregulated in ALS patients and validated five of them as a proof of concept. Importantly, we found that NRF2 deficient cells show a delayed clearance of stress granules induced by sodium arsenite, suggesting a crucial role for NRF2 in their dynamics.
Conclusions: NRF2 modulates stress granule dynamics, suggesting it as a promising therapeutic target to reinforce the expression of crucial genes related with RNA metabolism. Further studies are needed to fully understand these mechanisms and assess the potential of NRF2 modulation in ALS pathogensis.

Muhammet Karaman

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Muhammet Karaman is an associate professor in Molecular Biology and Genetic Department, Faculty of Sciences at the Kilis 7 Aralik University, Turkey. He has a doctorate at biochemistry. He's working on discovering interaction of protein between pathways and drug candidates for cancer treatment.

Disabling of Nrf2 activation via inhibition of NADPH metabolism-related antioxidant enzymes

Fatma Yapici, Muhammet Karaman

Background: In case of oxidative stress Nrf2 is activates and transits to the nucleus. In here, it binds to Antioxidant/Electrophile Response Element (ARE/EpRE) in the promoter regions of the target genes. Important antioxidant enzymes and ROS scavengers such as the thioredoxin and glutathione systems are expressed as a result of this process. Cancer cells in which reactive oxygen species are produced excessively compared to normal cells Nrf2 activation is induced. This is one of the most important defenses that ensures the survival of cancer cells. Inhibition of the antioxidant defense system induced by Nrf2 activation causes a decrease in the resistance of ca ncer cells to chemotherapeutic agents. For this purpose, new molecules were designed for the inhibition of the G6PD enzyme responsible for the production of NADPH, which is used as a reducing power source in the destruction of ROS, and the effects of these molecules on the activity of some NADPH-dependent antioxidant enzymes were analyzed by *in silico* methods.
Methods: Novel quinazolinone-imine derivatives were synthesized and their inhibitory effects on G6PD enzyme activity were determined. Then, the binding scores and modes of all molecules against Gr and TrxR1 enzymes were determined by molecular docking method.
Results: The findings obtained from kinetic studies had demonstrated that quinazolinone-imine derivative decreased G6PD enzyme activity at the nM level with IC_{50} values. It was determined that the G6PDInh-3 compound with an IC_{50} value of 48.71 nM exhibited the best G6PD inhibitor efficiency. The binding scores G6PDInh-3 compound were calculates as -8.1 kcal/mol, -10.0 kcal/mol, and -9.1 kcal/mol for hG6PD, hTrxr1, and hGr enzymes, respectively.
Conclusions: The synthesized inhibitors exhibit potent inhibitory properties against the enzymes that control the production of NADPH and the reduction process via NADPH. These compounds will cause inhibition of enzymes whose expression is induced by activation of Nrf2 and, accordingly, initiation of the apoptotic process in cancer cells. The properties of the quinazolinone-imine derivatives make them ideal drug candidates for chemotherapeutic treatment.

Andrea Mičurová

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I am a PhD student at the Centre of Experimental Medicine, Institute of Normal and Pathological Physiology, with a bachelor`s and master`s degree in biochemistry from Comenius University, Bratislava, Slovakia. I am mostly interested in cardiovascular research, antioxidant mechanisms, iron metabolism and its relation to NRF2, but as a very curious person I am also opened to learn as much as I can in other topics.

Different role of NRF2 in the liver and heart of rats exposed to polyethylene glycol-coated magnetite nanoparticles

Background: Iron oxide nanoparticles (IONs) are used in many areas, however, their usage is associated with negative side effects, mostly reactive oxygen species (ROS) production. It was shown that with elevated ROS and nitric oxide (NO), nuclear factor erythroid 2-related factor 2 (NRF2, encoded by *Nfe2l2* gene) translocation into the nucleus is elevated and it induces transcription of genes involved in antioxidant defence and genes involved in iron metabolism, such as ferritin (*Fth1), transferrin (Tf)* and ferroportin (*Fpn1*). Besides the role of NRF2, cellular iron metabolism is also controlled by iron-regulatory protein *(Irp1)*, which binds to iron-responsive element in *Fth1, divalent metal transporter (Dmt1) and transferrin receptor 1 (Tfr1)* mRNAs. **Methods:** We investigated the effect of single infusion of IONs (~30 nm, 1mg Fe/kg) 100 min post infusion in the liver and heart of Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). We determined ION- and biogenic iron levels (using SQUID magnetometry), superoxide and NO productions and expressions of selected genes involved in the regulation of iron metabolism, and their possible regulation by NRF2. **Results:** Saturation magnetisation (parameter of iron content) was significantly elevated in the liver but reduced in the heart of control SHR vs WKY. ION levels were lower in both tissues of SHR vs WKY. Elevated superoxide production was found only in the tissues of ION-treated WKY, while NO production was reduced in the liver of ION-treated SHR. Gene expression of *Nfe2l2 was* elevated in the liver of SHR vs WKY (main effect of genotype) and expressions *of Dmt1, Gpx4, Nos2, Nos3 and Sod2* correlated with *Nfe2l2,* but not with *Irp1*. In the hearts, gene expressions of *Nos2*, *Nos3*, *Sod1*, *Sod2*, *Fpn*, *Tf*, *Dmt1* and *Fth1* correlated with *Irp1* but not with *Nfe2l2*. **Conclusions:** Our results suggest differences in regulation of iron metabolism on gene level in the heart and liver. In the heart, expressions of various genes involved in iron metabolism seem to be regulated predominantly by iron content. In the liver, expressions of genes correlated with *Nfe2l2,* suggesting significant effect NRF2–mediated mechanisms in regulation of iron metabolism. Supported by VEGA 2/0157/21 and APVV-16-0263.

Marina Oskomić

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As a recently appointed PhD research assistant at the Laboratory for Protein Biochemistry and Molecular Modeling, my research is focused on protein interactions and the cellular responses to stress conditions. I hold a Master's degree in Molecular biotechnology and conducted my Master's thesis research in the Laboratory for Biochemistry. My research investigated the association between the RNA exosome protein complex, involved in RNA metabolism, and the cellular response to the antitumor drug 5-fluorouracil. Throughout my graduate and undergraduate studies, I gained extensive experience in utilizing nucleic-acid, protein, and in vivo-based techniques, as well as presenting my research at various professional conferences and competitions. I am particularly interested in further research on protein interactions, with a specific focus on the NRF2 protein. I am currently seeking a unique opportunity to deepen my knowledge of NRF2 protein interactors, which are crucial for developing targeted therapies that can manipulate NRF2 activity in a controlled and specific manner.

Protein interactions of DPP3 and their putative impact on NRF2-KEAP1 signaling

Background: DPP3 is a zinc metallopeptidase that cleaves dipeptides from the N-termini of 3-10 long peptides. DPP3 is a part of central human proteome and has a proposed role in the final stages of protein turnover in the cells, and putative role in the regulation of blood pressure and pain. DPP3 is also involved in the regulation of NRF2-KEAP1 oxidative stress response pathway through its interaction with KEAP1. KEAP1 is the only confirmed interactor of DPP3 and the interaction with KEAP1 is independent of DPP3 peptidase activity. In order to search for novel protein interactors of DPP3 we have employed SILAC-MS approach with HA-DPP3 as a bait in HEK293T cells and identified novel, putative interactors of DPP3. Currently, we are performing co-immunoprecipitation experiments in order to confirm selected interactions.
Methods: Co-immunoprecipitation (Co-IP), western blotting.
Results: Four biological replicates of SILAC-MS with HA-DPP3 as a bait in HEK293T cells were performed and more than 30 novel, putative interactors of DPP3 were identified. The best candidates were selected based on SILAC-MS ratio (quantity of the protein in DPP3 vs. empty vector transformed cells lysates) and physiological roles of prey proteins. We are currently performing co-IP experiments on endogenous proteins trying to confirm the selected interactions with DPP3-KEAP1 interaction as a positive control.

Patrícia Pavelková

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Patrícia Pavelková is currently starting her PhD. studies at the Faculty of Natural Sciences, Comenius University, Bratislava in the study program Animal Physiology. She is working on her PhD. thesis at the Institute for Heart Research, Centre of Experimental Medicine, Slovak Academy of Sciences (CEM SAS) focusing on molecular hydrogen and its effect on various cardiovascular diseases. She previously finished her master's degree in the Department of Genetics, Faculty of Natural Sciences, Comenius University.

The comparison of molecular hydrogen administration methods on radition-induced heart damage

Patrícia Pavelková, Barbora Kaločayová, Ján Slezák, Branislav Kura

Background: Oxidative stress is a common denominator of many cardiovascular diseases. Excessive production of free oxygen radicals (ROS) arises mainly due to the effects of various external sources, such as exposure to ionizing radiation. One of the possible treatment methods for eliminating ROS is the administration of antioxidants. Molecular hydrogen (H_2) is proving to be an effective strategy in treating the negative effects in many diseases.

Methods: In this study, we used 12-week-old male Wistar rats irradiated into mediastinum area by single dose of 10 Gy. These rats were divided in one control, one irradiated group with no treatment, and two experimental groups treated with H_2. One experimental group was treated with H_2 gas (3 x 30 min, 4% H_2) and the second group received H_2-rich water (3 x 3 mL, 2 ppm). We monitored the effects of H_2 2 and 9 days after irradiation.

Results: We observed significant changes in the oxidative stress (malondialdehyde, superoxide, glutathione peroxidase, superoxide dismutase), inflammatory parameters (NF-κB), and changes in the levels of the transcription factor Nrf2 in treated animals after irradiation. Administration of hydrogen in gaseous form significantly reduced these parameters to the level of non-irradiated animals.

Conclusions: Our results indicate that H_2 is an effective way to reduce the negative effects of radiation. We have observed that inhalation is a more suitable form of H_2 administration.

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Thomas Dixon

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Following the completion of A-Level chemistry, biology and mathematics at Sixth Form in England, I developed a keen interest in disease mechanisms and drug discovery. After completing my A-level studies, I began an integrated Master of Pharmacology course (with industrial placement), at the University of Bath. I graduated with a first class honours degree and a year of industrial experience working in Boehringer Ingelheim's immunology and respiratory disease research department in Germany. There, I became fascinated by the complexity of the mechanisms that underpin pulmonary fibrosis. This curiosity drove me to apply for a PhD position at the University of Dundee with Professor John Hayes, where I am currently investigating the reciprocal relationship between Nrf2 and TGF-β in the context of pulmonary fibrosis and how this may be exploitable.

Evaluation of the mechanisms by which activation of the transcription factor Nrf2 inhibits lung fibrosis

Background: Our hypothesis is that non-canonical TGF-β signalling leads to activation of kinases (JNK, $p38^{MAPK}$) that phosphorylate Ser residues in and/or flanking the DSGIS motif in the Neh6 domain of Nrf2, thereby priming Nrf2 for phosphorylation by GSK-3. Phosphorylation by GSK-3 then facilitates β-TrCP / cullin-1-mediated ubiquitylation of Nrf2 and its proteasomal degradation. Because Nrf2 activation supresses fibrogenesis, we envisage its downregulation by GSK-3 contributes to progression of fibrosis. As very little is known about this priming mechanism it is important to establish a robust cellular model to allow the mechanism by which TGF-β downregulates Nrf2 to be studied, and also to develop our understanding of how the priming process is triggered by other signalling pathways. **Methods:** A549 (adenocarcinomic human alveolar basal epithelial cells) grown in 10% FBS-containing DMEM media have been used as a model, since Keap1 is mutated and so constitutive levels of Nrf2 are high. Western blotting and qRT-PCR have been used study the effects of acute TGF-β treatment on Nrf2 protein and mRNA levels respectively. A recombinant protein comprising Neh6 and flanking residues (i.e., residues 290-410 of mNrf2, called $Neh6^{+}$) fused with GST has also been generated following cloning into pGEX-GST and expression of the resulting plasmid (pGEX-GST-$Neh6^{+}$) in E. coli and purification using a GSH-column. **Results:** At this stage, results are only preliminary, but appear to show a reduction of both Nrf2 protein and mRNA levels in A549 cells following TGF-β treatment**. I** am currently awaiting results from a kinase screen that should reveal kinases that phosphorylate Ser-335 and Ser-338 in the Neh6 domain. I will then validate these results using the GST-$Neh6^{+}$ fusion protein that I have already isolated, in addition to further Nrf2-based mini-proteins yet to be created. I also plan to study the effect of TGF-β on Nrf2 protein expression when cells are co-treated with inhibitors of the candidate kinases (JNK, $p38^{MAPK}$, and others). **Conclusions:** Showing that TGF-β treatment reduces Nrf2 expression in A549 cells is an important finding and gives me a model that I can use to continue to investigate the mechanism of β-TrCP-mediated ubiquitylation of Nrf2, triggered bv TGF-β. Furthermore, successful purification of $Neh6^{+}$ protein indicates that our existing protocol is valid and allows me to continue to generate new mini-proteins that will be used to help validate results from the kinase screen in the near future.

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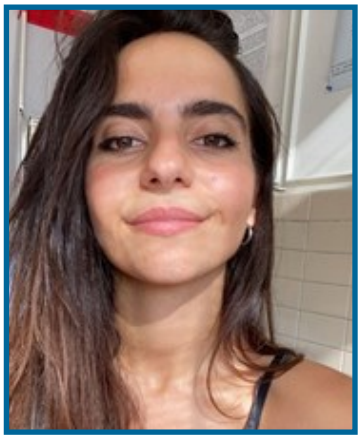

I began to develop a strong interest in research following my experimental thesis internship at Sapienza's Department of Pharmacology. After my Master's degree in Pharmaceutical Biotechnology at Sapienza, I started my PhD program in Biochemistry at the Department of Biochemical Sciences "A. Rossi Fanelli" of Sapienza. My work is mainly focused on the study of molecular mechanisms responsible for metabolic alterations in the neurodegenerative process, such as Down syndrome and Alzheimer disease.

Trisomy21 and aberrant BACH1/Nrf-2 axis: implications for neurodegeneration

Background: Several studies support the implication of oxidative stress (OS) in phenotypic changes in Down syndrome (DS) subjects. Mapping of Human Chromosome 21 (HSA21) has shown the involvement of several genes, such as SOD-1, BACH1, APP, CBR, and S100B, in reactive oxygen species (ROS) overproduction in DS subjects and in animal models. We focused our attention on BACH1, a transcription repressor that competes with the Keap1-Nrf-2-ARE complex and negatively regulates the Nrf-2-mediated antioxidant response. For this reason, we studied the role of BACH1 in the brain and its implication in the failure of antioxidant response in DS.
Methods: In this scenario, we investigated the BACH1/Nrf-2 dysregulation in human DS cells and animal models of the disorder: for human studies we analyzed lymphoblastoid cell lines (LCLs), whereas for animal studies we isolated hippocampal astrocytes and neurons from Ts2cje mice.
Results: Our results revealed that overexpression of BACH1 alters the BACH1/Nrf-2 ratio in the nucleus and impaired the transcriptional activation of antioxidant response genes, ultimately leading to the accumulation of oxidative damage.
Conclusions: Overall, our study supports the hypothesis that BACH-1 triplication in DS subjects plays a critical role in the alteration of redox homeostasis; therapeutic strategies able to restore Bach1/Nrf2 axis are currently under investigation in our laboratory.

Valentina Sophia Rumanova

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Our group from the Department of Animal Physiology and Ethology studies the effects of low-intensity light at night on the circadian system and the temporal organization of physiological processes.

Research interests: chronobiology, metabolism, neuroendocrinology, behaviour

Effects of dim light at night on daily rhythm in *Nrf2* expression and metabolism in rats

Background: Increasing light pollution (ALAN) has become part of our lives and is associated with cardio-metabolic diseases. The altered light:dark cycle can disrupt the circadian organisation of physiological processes, such as redox balance and metabolism. In our previous study, we showed disturbing effects of ALAN on redox balance in the kidney, manifested by upregulated *Nrf2 and Pgc1a levels.* The aim of this study was to evaluate the rhythmic expression of genes encoding the important transcription factor *(Nrf2)* and antioxidative enzymes (*Sod2, Hmox1*) in the liver of rats under ALAN. In addition, we analysed the effects of ALAN on metabolic rhythms, as oxidative stress is often linked with non-alcoholic fatty liver disease.
Methods: Adult male Wistar rats were kept either in 12:12 light:dark cycle (CTRL) or exposed to low-intensity light (~ 2 lx, ALAN) during the dark phase for 2 weeks. Blood and tissue samples (liver and adipose tissue) were collected in 4-hour intervals over 24 hours.
Results: In the liver, gene expression of *Sod2* displayed daily rhythm and *Hmox1* tended to be rhythmic. Hepatic *Nrf2* mRNA levels did not oscillate throughout the day. Exposure to ALAN eliminated the *Hmox1* rhythm, but *Nrf2* and *Sod2* were unaffected. On the other hand, we observed the loss of daily oscillations in behavioural parameters, plasma metabolites (glucose, triacylglycerols, cholesterol) and dysregulated rhythms of metabolic sensors (*Sirt1*, *Ppars*) that probably contributed to the chronodisruption of the various metabolic pathways.
Conclusions: ALAN did not affect *Nrf2* expression in the liver which could be explained by the short-term ALAN exposure and probable higher resistance of the liver to oxidative stress compared to the kidney. However, ALAN disrupted several daily rhythms in metabolism which can increase the risk of the diseases of civilization.

Iza Oblak

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I work as a young researcher at the Institute of Pathophysiology of the Faculty of Medicine at University of Ljubljana under supervision of Professor Irina Milisav. I am a second-year student on the Biomedicine Doctoral Programme. I have a master's degree in Biochemistry and a bachelor's degree in Cosmetic Science. Since my research work is in a field of stress responses, I believe it would be very useful for me to familiarize myself with the role of Nrf2, which is involved in various aspects of cellular and organismal defence against oxidative stress in non□communicable diseases. In my work, I mainly focus on liver cells, so a lecture on Nrf2 and liver diseases could help me understand the characteristic mechanisms related to the liver. I am just at the beginning of my work in the field of Nrf2, so I think this training course would provide me with a comprehensive knowledge of Nrf2, the master regulator of multiple cytoprotective responses.

NRF2 and drug-induced liver injury

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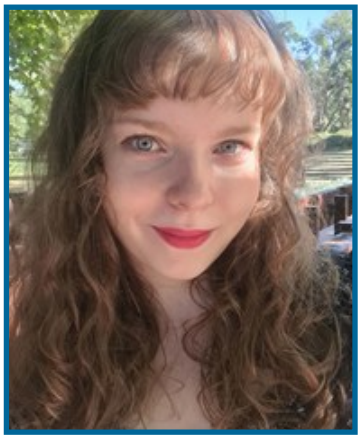
Oxidative stress is caused by various factors, such as ingestion of drugs, herbs, chemicals, and supplements, and it can lead to liver damage, like drug-induced liver injury (DILI), hepatic steatosis, etc. DILI is the leading cause of acute liver failure and liver transplantation in Western countries. While detection of the underlying mechanisms leading to potential drug hepatotoxicity is crucial and remains a major challenge, many studies have clarified that the Kelch-like ECH-associated protein 1-NFE2-related factor 2 (Keap1 -Nrf2) system, which is a crucial defense mechanism of cells and organisms in response to oxidative stress, is involved in the prevention and attenuation of liver injury. The nuclear factor-erythroid 2-related factor 2 (Nrf2) is involved in several aspects of cellular and organismal defense against oxidative stress. Its activation is triggered by oxidative or electrophilic stress and mediates the expression of cytoprotective genes by antioxidant responsive elements (ARE). Expression of cytoprotective genes provides a regulatory network of detoxification enzymes involved in antioxidant metabolism, intermediate metabolism of lipids, protein degradation, and regulation of inflammation. In this way Nrf2 is able to maintain the steady state of the internal environment responding to diverse forms of stress. We would like to investigate Nrf2 and its relation to non-communicable diseases in normal and stress-adapted cellular DILI models. Our work may contribute to the aims of the BenBedPhar Action, if there are differences in expression/localization of Nrf2 or its targets.



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PhD student at the Faculty of Natural Sciences, Comenius University in Bratislava. She is working under supervision of Dr. Szeiffová Bačová at the Institute for Heart Research, Centre of Experimental Medicine of the Slovak Academy of Sciences. The topic of her PhD thesis is: ,,Suppression of inflammation, its effect on pro-fibrotic signalling pathways in the heart, and protection against functional failure".

Role of oxidative stress in heart failure

Background: Heart failure (HF) is a pathophysiologic state, in which the heart fails to pump blood at a rate proportional to the requirements of the metabolizing tissues. One of the factors in the development of HF is an excessive accumulation of extracellular matrix in the heart, known as cardiac fibrosis, leading to myocardial electrical instability, and impairment of connexin-43 channels. Inflammation and oxidative stress significantly contribute to development of cardiac fibrosis by activating cardiac fibroblasts to trans-differentiate to myofibroblasts, which have a higher rate of migration, proliferation, and collagen production. Nuclear factor erythroid 2–related factor 2 (Nrf2) is a transcription factor which exhibits a major role in amplification of the antioxidant pathways. It may possess a potential for targeting oxidative stress–induced cardiovascular diseases including heart failure.
Methods: We will be monitoring signalling pathways of oxidative stress in the rodent model of heart failure, as well as protective effects of selected antioxidants – melatonin and molecular hydrogen. To achieve the aims of our study, we will use proteomic and microscopic methods.
Conclusions: Oxidative stress plays an important role in the pathophysiology of failing heart. Therefore it is relevant to monitor its signalling pathways as well as protective effect of substances with antioxidant properties, where Nrf2 can be implicated.

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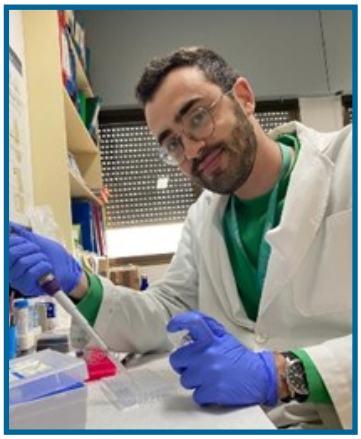

I enrolled in Biochemistry in 2015, where I found out that my research interest lied within the field of Neuroscience. Since then I have always conducted my research in the field of Alzheimer´s disease (AD), both in my undergraduate and master´s thesis. In the former I analysed the pharmacological potential of two serotoninergic analogs in AD and in the latter I studied behavioural tagging in a *knock-in* mouse model of AD. Upon completion, I returned to Madrid to work on my PhD thesis with the purpose of elucidating the role of NRF2 in synaptic maintenance and its possible therapeutical role in AD.

The role of NRF2 in synaptic homeostasis

Background: Despite intensive research, all lines of study have failed to develop an AD modifiying therapy, thus a new innovative approach is needed. In this study we will analyze the option to reinforce the connectivity of the remaining neurons in the AD brain targeting NRF2. NRF2 is a transcription factor responsible for activating a wide citoprotective programm in brain. We have previously characterized a new AD mouse model, combining amyloidopathy (*hAPP*^{V717I}) and tauopathy (*hTAU*^{P301L})with the presence (AT-NRF2-WT) or absence of NRF2 (AT-NRF2-KO). Interestingly, synaptic transmission in the neurons of the dentate gyrus was reduced in NRF2-deficient mice compared with wild-type littermattes (long-term potentiation analysis by electrophysiology).
Methods: Employing microarray technology, we have analyzed 25,000 transcripts in brain samples from these mice**.** To examine excitatory synaptic contacts, we evaluated the colocalization of vGlut1 and PSD95, pre- and postsynaptic markers respectively, both in brain slices and primary neuronal cultures. To study the molecular composition of these synapses, synaptosomes were isolated.
Results: The microarray data retrieved a significant dysregulation of 725 genes [more than 3-fold change; $p \leq 0.05$ (FDR/BH)]. Among these genes, we identified 16 clusters (formed by 122 genes) whose expression was altered by the absence of NRF2 and are related indirectly or directly to synapsis. Our findings revealed that the absence of NRF2 modified the molecular composition of the synapse, and caused alterations in the quantity and size of excitatory synaptic contacts. Relevantly, activation of NRF2 increased the number of excitatory synaptic contacts in primary neuronal cultures.
Conclusions: NRF2 deficiency modifies synaptic dynamics, providing a new avenue for exploring its potential as a therapeutic target for neurodegenerative diseases characterized by progressive synaptic loss, such as AD.

Hatice Esenkaya

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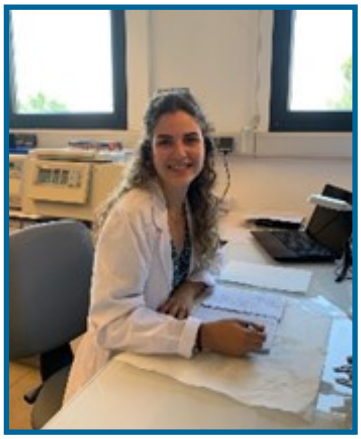
Recently graduated with a PhD from the Department of Molecular and Cell Biology at the University of Leicester, UK, supervised by Professor Ian Eperon and Dr Cyril Dominguez, and working on RNA splicing. My recent research was studying the effects of the G-quadruplex stabilising ligand GQC-05 on alternative splicing of Mcl-1 pre-mRNA. My interests are in pre-mRNA splicing and the interaction between spliceosome proteins and RNA. I am particularly interested in how their interaction affect the outcome of alternative splicing. and if their interactions can be altered, using a variety of methods, to see if the splicing pattern can be shifted to obtain more favourable isoform.

Investigating the effects of G-quadruplex structures in NRF2

Background: Nuclear factor erythroid 2-related factor 2 (NRF2) is encoded by human *NFE2L2* gene and has a role as a transcription factor. This family of proteins regulate expression of antioxidant proteins, Phase II detoxification enzymes and other cytoplasmic enzymes. Studies have shown that the activation of NRF2 is a potential therapeutic target for inflammatory diseases, however inhibition of NRF2 also benefits due to its resistance to some tumour types. The sequence of NRF2 gene around the promoter region sites and the 5' untranslated region (UTR) of NFR2 mRNA has been shown to contain a number of G tracts and C tracts. Based on bioinformatics analysis some of these regions have the potential to form G-quadruplexes (G4s), four-stranded structures formed by the interaction of such G rich sequences. G4s may affect various DNA and RNA processing reactions, including telomere lengthening to DNA replication, transcription, translation, and splicing. Since G4s are located in guanine rich regions, telomeric regions, gene promoters, and 5' UTRs, their formation could be a key regulator of aging and age-related degeneration, along with antioxidant processes such as activation of NRF2. Moreover, the existence of G4 structures in NRF2 mRNA may affect NRF2 protein translation. Therefore, exploration and modulating of G4 formation in NRF2 DNA or RNA and their interactions with small molecule ligands has been seen as a possible route to regulating antioxidant system and other pathological systems.
Methods: QUADPARSER, CD (circular dichroism)in the presence of KCl or LiCl.
Results: The preliminary data shows potential existence of G4 in NRF2.
Conclusions: It is shown that G4s are important for the regulation of transcription and translation. The preliminary data shows that NRF2 has potential to form these structures, and could be manipulated to activate or deactivate NRF2 gene by the use of G4 stabilisers. Further investigations are required to confirm whether NRF2 contain G4 stuctures, and to charcterise their role in transcription an translation processes.



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I studied a degree in Biology at the University of Córdoba from 2015-2019. Afterwards, and to continue in the reasearch field I took a Master's degree in Biotechnology at the same university. I carried out my Master's thesis at the renal and vascular physiopathology laboratory in the Maimonides Institute of Biomedical Research (IMIBIC), where I'm currently working as a PhD student.

Nrf2 in acute kidney injury associated with rhabdomyolysis

Background: Rhabdomyolysis is a serious clinical syndrome caused by skeletal muscle damage that may lead to acute kidney injury (AKI). There is no specific treatment for rhabdomyolysis-associated AKI, only supportive care. For that reason, it is necessary to identify novel pathways involved in this pathological setting. In this line, several articles have suggested the role of Nrf2 in rhabdomyolysis, although the role of this transcription factor has not been fully analyzed.
Methods: We first performed an *in silico* approximation based on data previously published. We analyzed the kidney transcriptome obtained through RNA-seq from an experimental mouse model of rhabdomyolysis. To confirm these findings, we developed an experimental model of massive muscle injury that promoted AKI and analyzed whether induction of Nrf2 may be effective against rhabdomyolysis-mediated renal injury.
Results: Principal component analysis showed that the biological replicates clustered separately between groups (saline vs rhabdomyolysis), indicating different transcriptional profiles. Pathway enrichment analysis indicated dysregulation of the Nrf2 pathway. Thus, we identified 77 differentially expressed genes (DEG) related to the Nrf2 pathway, including 64 upregulated and 13 downregulated genes. In addition, renal tissue from mice with rhabdomyolysis showed alterations in oxidative stress, cell death (apoptosis regulation), and inflammation (NF-kappaB signaling, Myd88 pathway, among others) associated with Nrf2 pathway. *In vivo* and *in vitro* essays showed Nrf2 inducers sulforaphane and curcumin increased Klotho expression, an anti-aging protein expressed by the kidney and augmented HO-1.
Conclusions: Nrf2 is involved in rhabdomyolysis-associated AKI. Strategies to upregulate Nrf2 pathway may be potentially useful to decrease adverse consequences of rhabdomyolysis-AKI.

Michal Kluknavský

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I completed my PhD studies at the Comenius University in Bratislava, Slovakia. My PhD studies were focused on the investigation of the cardiovascular effects of cacao flavanol (-)-epicatechin in prehypertensive and hypertensive rats. Currently I am postdoctoral researcher in the Department of experimental hypertension, CEM SAS, Bratislava and I am involved in the research of NRF2 mediated mechanisms in conditions of chronic and acute stress in rats with various genetic predisposition to hypertension.

ACE2 Inhibitor MLN-4760 elevates expression of *Nfe2l2* and antioxidant genes in the brainstem of spontaneously hypertensive rats

Background: Numerous studies showed that angiotensin-(1-7) acts as an antihypertensive and antioxidant agent. Reduced bioavailability of angiotensin-(1-7) due to inhibition of angiotensin-converting enzyme 2 (ACE2) may contribute to increased mortality in hypertensive individuals. However, effects of ACE2 inhibitor MLN-4760 in brain remain unknown. **Methods:** We investigated the selected behavioural and hemodynamic parameters in spontaneously hypertensive rats (SHRs) after a 2-week s.c. infusion of MLN-4760 (dose 1 mg/kg/day). The biochemical and molecular effects of MLN-4760, including the effects on *Nfe2l2* gene expression (encoding NRF2), were investigated in blood plasma and the brainstem. **Results:** MLN-4760 did no alter hemodynamic and behavioural parameters compared to vehicle-treated controls. However, MLN-4760 increased hydrogen sulfide (H_2S) level in plasma and total nitric oxide (NO) synthase activity and conjugated dienes levels (marker of oxidative damage) in the brainstem. Increased NO synthase activity correlated positively with gene expression of *Nos3* while plasma H_2S levels correlated positively with gene expressions of H_2S-producing enzymes encoded by *Mpst*, *Cth* and *Cbs*. MLN-4760 administration increased gene expression of *Ace2, Sod1, Sod2, Gpx4* and *Hmox1*, which positively correlated with expression of *Nfe2l2*. However, MLN-4760 had no effect on expression of *Il1b*, *Tnf*, *Ptgs1*, *Ptgs2* and *Pparg genes*. **Conclusions:** Collectively, MLN-4760 did not exacerbate pre-existing hypertension and behavioural hyperactivity/anxiety in SHRs. MLN-4760-induced oxidative damage in brainstem was associated with activation of NO- and H_2S-mediated compensatory mechanisms and with increased gene expression of antioxidant, NO- and H_2S-producing enzymes that all correlated positively with elevated *Nfe2l2* expression.

Study was supported by the grant No. PP-COVID-20-0043.

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I am a Ph.D. student in Medical Neurosciences at the Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Bratislava, Slovakia. Previously I studied biochemistry at the Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia. My research focuses on nutritional intervention and physical activity in relation to aging.

The effect of a combined intervention (omega-3 fatty acids and physical activity) on functional parameters of sarcopenia and oxidative stress in aged rats

Background: Aging causes degenerative processes in the organism and can lead to various civilization diseases that affect the population. It was confirmed, that omega-3 fatty acids and exercise can alleviate oxidative stress through nuclear factor-erythroid-2-related factor 2 (Nrf2) regulation which can induce transcription of antioxidant enzymes as SOD, GPx and CAT, and thus achieving better health during the process of aging.
Methods: We determined the basic biochemical and anthropometrical and functional parameters of sarcopenia (body weight, step length, muscle index) and the activities of antioxidant enzymes in erythrocyte hemolysate. We determined the total antioxidant capacity of plasma using two methods: Trolox equivalent antioxidant capacity (TEAC) and Ferrum reducing ability of plasma (FRAP). In the hemolysate of erythrocytes, we determined the activity of antioxidant enzymes - superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). We determined SOD and GPx activities with commercial kits (Sigma-Aldrich; USA and Cayman Chemicals, USA) and CAT activity according to Bergmeyer.
Results: Our results showed that old rats have lower plasma urea concentration and higher plasma total cholesterol values, impaired sarcopenia functional parameters, and plasma sarcopenia-related parameters. Physical activity alone improved functional parameters of sarcopenia, increased GPx activity and TEAC. A lower dose of omega-3 FA in combination with exercise reduced CAT activity and increased TEAC. A higher dose of omega-3 FA in combination with exercise increased step length, reduced CAT activity and increased TEAC.
Conclusions: Combined intervention has the potential to have a positive effect on the functional parameters of sarcopenia in old rats, as well as on the antioxidant protection of the organism.

Louisa Watt

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I am a first year PhD student at the University of Dundee in the Dinkova-Kostova lab (started in October 2022). My project investigates the therapeutic potential of pharmacological activation of NRF2 for liver fibrosis. The overall goal of my project is to examine the anti-fibrogenic potential of the cyanoenone triterpenoid RTA-405, a tool compound closely related to RTA-408 (also known as Omaveloxolone and SKYCLARYS™), which was recently approved by the United States Food and Drug Administration for the treatment of Friedreich's ataxia. My research interests include drug discovery, innovative models of disease and the complex nature of NRF2 and its context dependence. I would like to learn more about the relationship between NRF2 and cancer, and how NRF2 activation could be targetted specifically to the liver.

During this initial stage of my PhD, I have been mainly familiarising myself with the fields of NRF2 biology and liver fibrosis and learning basic laboratory techniques, and do not have any conclusive results from my project yet to share with the NRF2 community. Nonetheless, I am keen to learn and interact with other researchers in the NRF2 field, and would be most grateful if I am given the opportunity to participate in this summer school.

ACKNOWLEDGEMENTS

BenBedPhar Training School 2023
NRF2 in noncommunicable diseases: from bench to bedside

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