Program Istanbul
Thursday 9th

14:00 – 14:15 Welcome and Introduction Kemal Yelekci, Gina Manda, and Antonio Cuadrado

Session 1 Chairs: Antonio Cuadrado and **Gina Manda**
14:15 – 15:00 Plenary Lecture: The KEAP1-NRF2 Pathway: From Cancer Chemoprevention to Cancer Progression. **Masayuki Yamamoto** (Tohoku University, Tohoku Medical Megabank Organization, Sendai, Japan)
15:00 – 15:15 Obituary Thomas Kensler. **Dionysios V. Chartoumpekis**
15:15- 15:45 KEAP1-driven Dysfunction of Aging Endothelial Cells. Milena Cichoń, Aleksandra Kopacz, Damian Klóska, Alicja Józkowicz, and **Anna Grochot-Przęczek**
15:45- 16:15 Nrf2 modulates inflammation resolution by activating phagocytosis of apoptotic cells and biosynthesis of specialized-pro resolving mediators. **Erdi Sozen**, Young-Joon Surh, and Nesrin Kartal Ozer
16:15 – 16:45 Oxidative Stress Response Failure in HCC Development. Manuela Hidalgo, Yolanda Olmos, and **María Monsalve**

16:45 – 17:15 Coffee and Poster viewing

Session 2 Chairs: Anna Grochot-Przeczek and Isabel Lastres-Becker
17:15 – 17:45 Role of NRF2 in Reprogramming Glucose Metabolism in Hepatocellular Carcinoma. **Young-Joon Surh**
17:45 – 18:15 Aberrant BACH1/Nrf2 Axis in Down Syndrome Brain Contributes to Early Onset Alzheimer-like Neuropathology. Sara Pagnotta, Antonella Tramutola, Eugenio Barone, Fabio di Domenico, and **Marzia Perluigi**
18:15 – 18:45 Screening Natural Products for the Identification of NRF2 Activity Modulators. Despoina D Gianniou, Ioanna Kentroti, and **Ioannis P. Trougakos**
18:45 – 19:15 Identification of Novel Keap1 Inhibitors Using Machine Learning, Molecular Modeling and Molecular Dynamics Simulations. **Kemal Yelekçi**, Alp Eren Kazar, and Ceren Cebeci

Friday 10th

Session 3 Chairs: Ana I. Rojo and Albena Dinkova-Kostova
9:00 – 9:30 Activation of Nrf2 Signaling and Inflammation Reduction by Dietary Broccoli Sprouts in Cafeteria Diet-Fed Mice. Marian V. Ivanochko, Maria M. Bayliak, Dmytro V. Gospodaryov, **Volodymyr I. Lushchak**
9:30 – 10.00 Towards (marine) Nutraceutical based Ferroptosis Intervention Strategies to Support Aging Healthcare. **Wim Vanden Berghe**

10:00- 10:30 NRF2 Signaling Emerges as a Key Mediator in the Molecular Interplay between Defective Proteostasis and Metabolic Dysfunction in Neurodegeneration. **Fabio Di Domenico**

10:30-11:00 Pharmacological Inhibition of Nrf2 as a Strategy to Overcome Therapy Resistance in Cancer. **Srđan Bjedov**

11:00 – 11:30 Coffee and Poster viewing

11:30 – 12:00 Sex-Specific Differences in NRF2-Driven Stress Responses and Autophagy in MASLD and MASH Models: Toward Tailored Therapeutic Approaches. Marta Alegret, Núria Roglans, Jianing Zhou Wu, Yanhao Qiu, Elisabetta Profumo, Alessandra Berry, Letizia Giona, Luciano Saso, and **Brigitta Buttari**

12:00 – 12:30 NRF2 as a Key Regulator in Intestinal Inflammation Induced by Silver Nanoparticles and its Modulation with Quercetin. Adelaide Sousa, Inês Santos, Rui Fernandes, Sofia Pacheco, Félix Carvalho, Eduarda Fernandes, and **Marisa Freitas**

12:30 - 13:00 Repurposing Omaveloxolone as a Potential Therapeutic Approach for Early Age-Related Macular Degeneration. **Ana S. Falcão**, Margarida Pedro, Shuvajit Rakshit, Luisa Lemos, Pedro Antas, Sandra Tenreiro, and Miguel C. Seabra

13:00 - 13:30 NRF2 Induction Coupled with Cellular Delivery of Carbon Monoxide for Counteracting Inflammatory Conditions. **Roberta Foresti,** Roberto Motterlini

13:30 – 14:30 Lunch and Poster viewing

Session 4 Chairs: Christina Morgenstern and Silvia Giordani

14:30 – 15:00 Dissecting the Protein Interactome of Wild Type And Lung Cancer Associated Gain-of-Function Mutant Forms of NRF2. **Anna-Liisa Levonen**

15:00 – 15:30 Dual Modulation of NRF2 by KEAP1 and GSK3: A Shared Therapeutic Axis in Alzheimer's Disease and MASH. Raquel Fernández-Ginés, Marta Olazabal, José Jiménez-Villegas, Daniel Carnicero Senabre, Ángel J. García-Yagüe, Ana I. Rojo, and **Antonio Cuadrado**

15:30 – 16:00 Targeting NF-κB–Mediated Neuroinflammation to Recalibrate NRF2 Signaling and Mitigate Oxidative and Ferroptotic Stress in Parkinson's Disease. Lilia A. Smith, Ignacio Silva-Llanes, Enrique Madruga, Daniel Flores-Téllez, Virginia Solar-Fernández, Alfonso García-Rubia, Carmen Gil, Ana Martínez, and **Isabel Lastres-Becker**

16:00 – 16:30 Nrf2 Signaling as a Mediator/ Enhancer of Statin- and SGLT2 Inhibitor Benefits in Metabolic Disease. Eleftherios Bochalis, and **Dionysios V. Chartoumpekis**

16:30 - 17:00 Coffee break and working group meeting

Session 5 Chairs: Santiago Cuevas and **Irina Milisav**

17:00-17:30 Nrf2 in MASH; Potential Role of Lipid Droplet Maturation. Bengu Cetinkaya, Tugce Demirel-Yalciner, and **Nesrin Kartal Ozer**

17:30-18:00 The Thyroid as a Sentinel and Target Organ in Keap1/Nrf2 Pathway Activation: Lessons from Animal Models and Human Studies, and Implications for Nrf2-Targeted

Therapies. Panos G. Ziros, Georgios Psarias, Dionysios V. Chartoumpekis, and **Gerasimos P. Sykiotis**

18:00-18:30 Nrf2 is a Transcriptional Regulator of CD5L. Sharadha Dayalan Naidu, Elena V. Knatko, and **Albena T. Dinkova-Kostova**

18:30-19:00 Vascular Effects of Dimethyl Fumarate in Male Hereditary Hypertriglyceridemic Rats Exposed to Social Stress. **Iveta Bernatova**, Peter Balis, Aybuke Bozkurt, Andrea Micurova, and Michal Kluknavsky

19:00 – 19:15 Farewell Kemal Yelekci, Gina Manda, and Antonio Cuadrado

POSTERS

1. Nrf2 modulates immune responses in sepsis by regulating PANoptosis patterns in PBMCs. **Qian Zhang**

2. Design and validation of a SIMOA platform for the measurement of Nrf2 levels. Aysen Cotuk, Ender Avci, Burak Ibrahim Arioz, **Sermin Genc**, and Sibel Kalyoncu

3. Heme oxygenase-1 does not affect formation of stress granules in iPS cells. Jan Paczesniak, Patryk Chudy, Wojciech Krzeptowski, Witold Nowak, Jakub Kochan, Alicja Jozkowicz, and **Anna Grochot-Przeczek**

4. The transcription factor GATA4 is expressed in liver progenitor cells and confers protection against epithelial-mesenchymal transition. Laura Villamayor, Florian Segel, Noelia Arroyo, Elena del Fresno, Pedro M. Rodrigues, Malgorzata Milkiewicz7, Piotr Milkiewicz, Jesús M. Banales, Anabel Rojas, Águeda González-Rodríguez, and **Ángela M. Valverde**

5. Kino2omics integration platform bridging the genotype and phenotype gap in precision medicine. İkbal Agah İNCE, **Duru Tuncer**, Herald Berghmans, Steven Van Laere, and Wim Vanden Berghe

6. Structure-based discovery and optimization of KEAP1–NRF2 inhibitors. **Pınar Siyah**

7. Update on therapeutic strategies targeting nrf2 pathway modulation in kidney diseases. Celia Arias, Maria Jose Caballero, and **Santiago Cuevas**

8. Development of bile acid-based compounds as dual nrf2 activators and fxr modulators. Sergej Cvijić, **Srđan Bjedov**

9. Oxidative activity of cancer-selective carbon dots in vitro under physiological oxygen levels. Yingru Zhou, Francesco Calzaferri, Fan Yang , Giovanni E. Mann, and **Silvia Giordani**

10. NRF2 isoform 2 reveals additional level of NRF2 regulation. Zuzanna Urban-Wójciuk, Alicja Dziadosz-Brzezińska, Sara Kusinski, Maciej Cieśla, and **Alicja Sznarkowska**

11. Exploring the Role of DMF-driven NRF2 Activation in Lysosomal Function and Autophagy in RPE Cells. Margarida L. Pedro, **Ana S. Falcão**, Sandra Tenreiro, and Miguel C. Seabra

12. NetRF2: network-based analysis of tissue- and cell-specific targets and interactions of NRF2. **Ozlen Konu**, Rana Acar, Aida Rezaei, Muazzez Celebi, and Eren Kumak

13. Combining stress response augmentation and reductive stress relief through mild hypothermia. K.-L. Grassman, H. Vellama, F.M. Sirkel, T. Jagomäe, Rando Porosk, L. Tarve, T. Visnapuu, R. Reimets, C.A. Hundahl, E. Vasar, H. Luuk, and **K.-L. Eskla**

14. NRF2 Transcription Factor: Sculpting Synaptic Lipids for Brain Health.

Daniel Carnicero-Senabre, Mariana A Barata, José Jiménez-Villegas, Cláudia Guimas Almeida, Antonio Cuadrado, and **Ana I. Rojo**

15. NRF2 activators for treatment of neurodegeneration with brain iron accumulation. **Anton Terasmaa**, Rutt Taba, and Tuuli Käämbre

16. Chronic rhinosinusitis & NRF2: Inflammation, Oxidative Stress, and Biomarker Horizons. **Christina Morgenstern**

17. Endogenous Nrf2-mediated oxidative stress response is not activated in mouse models of Crohn's Disease. **Dina Dikovskaya**, and Mahima Swamy

18. NRF2-independent role of KEAP1 in actin cytoskeleton regulation. **Marina Oskomić**, Nikolina Stojanović, Andreja Ambriović-Ristov, and Mihaela Matovina

19. Advancements of young researchers within BenBedPhar COST Action on NRF2-targeted knowledge: Refining Redox Regulation for Therapeutic Applications. Krume Bogevski, **Viktorija Maksimova**

20. NRF2 and arachidonic acid mediators in neurodegenerative diseases. **Malvina Hoxha**

21. Cholesterol-induced lipid accumulation in hepatocytes: Insights from NRF2 Modulation. Bengu Cetinkaya, Tugce Demirel-Yalciner, Burak Emre Gunduz, and **Nesrin Kartal-Ozer**

22. Nrf2 knockout mice as a tool to study CNS autoimmunity. **Đorđe Miljković**

23. Kyn-CKA engages KEAP1 Cys151 to reprogramme macrophage inflammation via NRF2. Jialin Feng, Mara Carreno, Masayuki Yamamoto, Dario Vitturi and **Albena Dinkova-Kostova**

24. Differential sensitivity of breast cancer cell lines to H_2O_2-induced oxidative stress: roles of AMPK and the NRF2 pathway. Monika Mlinarić, Ivan Lučić, Ana Čipak Gašparović, and **Lidija Milković**

25. Dimethyl fumarate decreases plasma triglyceride level in female hypertriglyceridemic rats exposed to chronic psychosocial stress. **Mičurová A**, Kluknavský M, and Iveta Bernátová

26. Mechanistic insights into Garcinoic Acid's regulation of cholesterol-induced steatosis: role of NRF2/HO-1 Axis. Tuğçe Demirel-Yalçıner, Bengü Çetinkaya, Erdi Sözen, and Nesrin Kartal Ozer

27. DPP3 Knockdown impairs cell migration and cell cycle progression in HeLa cells. Lea Barbarić, Marina Oskomić, Anđela Horvat, Katja Ester, Ana Tomašić Paić, Nikolina Stojanović, and **Mihaela Matovina**

28. Pin1-Nrf2 Axis: characterization of interaction, pharmacological inhibitors, preliminary implications from endothelial aging models. Adem Ozleyen, Gizem Nur Duran, Mehmet Özbil, Serhat Donmez, Richard G Doveston, Milena Cichon, Anna Grochot-Przeczek, and **Tugba Boyunegmez Tumer**

29. Cardioprotective role of sirtuin1-deacetylase and Nrf2 axis in high-sucrose-diet-induced metabolic syndrome mouse. Leila Aryan, Suatnur Şık, Fırat Akat, Buşranur Parlak, Yusra nur Parlak, and **Erkan Tuncay**

30. In silico design of investigational drugs targeting ERα and BRCA1 proteins for breast cancer therapy. Melek Ezo Polatlı, and Kemal Yelekci

31. NRF2-activating agents exert antioxidant effects by enhancing endogenous H_2S formation. Emine Nur Ozbek, Zeynep Elif Yesilyurt Dirican, Ilayda Okumus, Medine Makal, Yiğitcan Şar, Ebru Arioglu Inan, and **Gunay Yetik-Anacak**

32. Phytochemical characterization of *Rhodiola rosea* L and its therapeutic effect on Alzheimer's disease. Aina Bellver-Sanchis, Christian G. Ferré, **Andrey S. Marchev**

33. Nox4 is involved in acute kidney injury associated to intravascular hemolysis. Cristina García-Caballero, Melania Guerrero-Hue, Mercedes Vallejo-Mudarra, Alejandra Palomino Antolin, Celine Decouty-Pérez, Luz Marina Sánchez-Mendoza, José Manuel Villalba, José Antonio González-Reyes, Lucas Opazo-Rios, Cristina Vázquez-Carballo, Carmen Herencia, Fernando Leiva-Cepas, Isabel Cortegano, Belén de Andrés, Jesús Egido, Javier Egea, Juan Antonio Moreno.

ABSTRACTS

O1: The KEAP1-NRF2 Pathway: From Cancer Chemoprevention to Cancer Progression
Masayuki Yamamoto
Tohoku University, Tohoku Medical Megabank Organization

We have identified the KEAP1-NRF2 system, which senses environmental stresses and activate cellular defense responses. NRF2 is a transcription factor that plays a crucial role in the coordinated expression of cellular defense enzymes against oxidative and electrophilic stresses. KEAP1 acts as a sensor for these stresses and continuously promotes the degradation of NRF2. *Nrf2* gene knockout animals are highly sensitive to a wide variety of toxic electrophiles and reactive oxygen species, whereas *Keap1* gene knockdown animals exhibit a gain-of-function phenotype characterized by enhanced NRF2-mediated cytoprotection. Genetic as well as pharmacological inductions of NRF2 protect our body from the oxidative and electrophilic injury. We have demonstrated the benefits of NRF2 activation in various disease models. Notable examples of the activity include the cancer chemoprevention and protection against space-related stresses. In contrast, cancer cells frequently hijack NRF2 activity, leading to NRF2-hyperactivated (or -addicted) cancers. Such NRF2-addicted cancers are refractory to standard radio-chemotherapy and are associated with poor prognosis. Recent structure-function analyses of KEAP1 have advanced our understanding of this system and opened the way for developing NRF2-activating cytoprotective agents as well as NRF2-inhibiting anti-cancer drugs. Research on the KEAP1-NRF2 system is also expanding into human biology utilizing the Tohoku Medical Megabank cohort study and into space mouse biology utilizing the International Space Station. Both historical contexts and recent advancements in the KEAP1-NRF2 field will be discussed.

O2: Title: In Memoriam: Thomas W. Kensler, PhD (1948–2025)
Dionysios V. Chartoumpekis
Division of Endocrinology, Department of Internal Medicine, School of Medicine, University of Patras, Greece

It is with deep sadness that I share the passing of Thomas Wells Kensler, PhD, professor emeritus and pioneering cancer prevention researcher, who died on July 11, 2025, at the age of 76 following a hiking accident on Mont Blanc in France. Born in New York City in 1948, Tom grew up in Lexington, Massachusetts, attended Buckingham Browne & Nichols School, earned an AB in biology from Hamilton College, and completed his PhD in toxicological

sciences at MIT in 1976. Following postdoctoral training at the University of Wisconsin and the National Cancer Institute, Tom joined the faculty of the Johns Hopkins Bloomberg School of Public Health in 1980, where he rose to professor and directed the Division of Toxicological Sciences. He later held appointments at the University of Pittsburgh and the Fred Hutchinson Cancer Center in Seattle, retiring in 2024.Renowned for his groundbreaking research in cancer chemoprevention, including nutrition-based strategies such as broccoli sprout interventions in China, Tom received many honors. Yet he valued most his role as a mentor. I was fortunate to be both his mentee and friend. His open-mindedness and example showed me how to pursue basic research on the Keap1/Nrf2 pathway while also striving toward clinical impact.
A passionate mountaineer and kayaker, Tom explored all seven continents and every U.S. state. He is survived by his wife of 43 years, Dr. Nancy E. Davidson, their children Kevin and Caroline, and extended family. Tom will be deeply missed, and for me, he will always be an inspiration in science and in life.

O3: KEAP1-driven Dysfunction of Aging Endothelial Cell

Milena Cichoń, Aleksandra Kopacz, Damian Klóska, Alicja Józkowicz, Anna Grochot-Przęczek

Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Medical Biotechnology, Kraków, Poland

Cardiovascular diseases (CVDs) are the leading cause of death globally. Endothelial cells (ECs) that line the inner surface of blood and lymph vessels pay critical role in maintaining healthy phenotype of vasculature. Aging dysregulates function of ECs which contributes to a higher risk of CVDs in the elderly. Our data suggest that NRF2 and KEAP1 can be involved in this process.
KEAP1 (Kelch-like ECH-associated protein 1) is a redox-sensitive repressor of NRF2 (NFE2L2 – nuclear factor (erythroid-derived 2)-like 2) – a transcription factor that mediates the protective response against oxidative stress. But, as we recently proposed, KEAP1 can act independently from NRF2 and along with nitric oxide synthase (NOS) and transnitrosating protein GAPDH be involved in the formation of S-nitrosothiols, leading to changes in protein function. In NRF2-deficient ECs this leads to the accumulation of protein aggregates, loss of function and premature senescence. Our data show that with age NRF2 level decreases in ECs, which suggests that the same dysfunctional phenotype of ECs can be occurring in physiological aging. Therefore, we study the relation between protein aggregation and cell function in young and aged human-derived primary endothelial cells with the possible involvement of S-nitrosation mediated by KEAP1/NOS/GAPDH SNO complex. Our results indicate that a higher level of protein aggregates appears with age along with the impairment of function, which can be restored by the modulation of KEAP1. This suggests a critical NRF2-independent role of this protein in ECs aging.

O4: Nrf2 Modulates Inflammation Resolution by Activating Phagocytosis of Apoptotic Cells and Biosynthesis of Specialized-Pro Resolving Mediators

Erdi Sozen1, Young-Joon Surh2, Nesrin Kartal Ozer 3,4

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3Department of Biochemistry, Faculty of Medicine, Uskudar University, Istanbul, Turkey
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Department of Biochemistry, Faculty of Medicine, Marmara University, Istanbul, Turkey
Billions of cells involved in immune response undergo apoptosis, and their efficient removal is crucial for resolving inflammation and maintaining homeostasis. Therefore, the phagocytic engulfment of apoptotic cells, particularly neutrophils by macrophages, known as efferocytosis, is crucial in preventing secondary necrosis and promoting tissue repair. Nrf2 is one of the well-studied transcription factors that holds a crucial impact in chemoresistance and cellular defence by enhancing various antioxidant and anti-inflammatory enzymes, including heme oxygenase-1 (HO-1). Although the Nrf2 has shown to enhance anti-inflammatory mechanisms, there is little information about their involvement in inflammation resolution, especially efferocytosis and specialized-pro resolving mediators (SPMs). In this direction, we aimed to evaluate the impact of Nrf2/HO-1 signaling in inflammation resolution, with a special focus on efferocytosis activity and SPM biosynthesis. First, bone marrow cells of C57BL/6 mice were collected and differentiation into macrophages was validated by both flow cytometry and confocal microscopy. Garcinoic acid, a natural analogue of delta-tocotrienol (δ-T3) derivative, and 17-oxo-DHA, an electrophilic metabolite of docosahexaenoic acid (DHA), was administrated and enhanced efferocytosis activity was observed, together with altered SPM levels. To further explore the role of Nrf2/HO-1 signaling, another set of macrophages were exposed to gene manupulation of Nrf2 or pharmacological inhibitin of HO-1 and efferocytosis activity was reduced. However, either Nrf2 silencing or HO-1 inhibition decreased SPM levels. Our findings indicate that Nrf2/HO-1 signaling stimulates efferocytosis and promotes SPM bioynthesis in garcinoic acid and 17-oxo-DHA administrated primary macrophages. Supported by The Scientific And Technological Research Council of Turkey (TUBITAK) 221N049.

O5: Oxidative stress response failure in HCC development
Manuela Hidalgo, Yolanda Olmos, María Monsalve
Instituto de Investigaciones Biomédicas Sols-Morreale (CSIC-UAM)
Context: Liver steatosis is a highly prevalent condition associated with the metabolic syndrome that can progress to Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), that can, in turn, lead to the development of cirrhosis and Hepatocellular Carcinoma (HCC). The determinant features of this progression are unclear and the condition does not have effective treatment options. In view of the well described presence of mitochondrial oxidative stress associated to MASLD, we decided to evaluate the contribution of impaired mitochondrial function to MASLD progression to HCC. Methodology Approach: We compared disease progression in WT and PGC-1 α KO mice. PGC-1α is a master regulator of oxidative metabolism and PGC-1α deficient mice are a mouse model for the loss of metabolic plasticity and mitochondrial oxidative stress. WT and KO 12 week-old mice were fed with normal or high-fat diet, and treated or not with

teratogens (T) for 24 weeks. Results: Weight gain follow up showed that animals treated with HFD+T gained less weight that single treated animals, and the effect was significantly more marked in KO mice. Histology analysis of liver samples showed that liver fat accumulation was, as previously described lower in KO mice tan in WTs but more importantly HFD+T mice had lower levels of fat accumulation than HFD mice and again the effect was higher in KO mice. Disease development was confirmed by evaluation of tumor burden, that was significantly higher in KO mice than in WT mice. Enhanced tumor burden in double treated KO mice was not associated to higher proliferation rates, nor higher levels of fibrosis, but with reduced cell death rates despite increased oxidative stress levels related to failure to induce antioxidant and detoxification systems in KO mice. Conclusions: These results support the hypothesis that mitochondrial dysfunction plays a key role in the HCC and indicate that PGC-1α deficiency is linked to higher levels of oxidative stress and failure to induce cell death pathways leading to HCC.

O6: Role of NRF2 in Reprogramming Glucose Metabolism in Hepatocellular Carcinoma

Young-Joon Surh

College of Pharmacy, Seoul National University

Hypoxia-inducible factor-1α (HIF-1α) and nuclear factor erythroid 2-related factor 2 (NRF2) are constitutively overactivated in hepatocellular carcinoma (HCC). In the present study, we investigated their possible complementary roles in growth and progression of HCC and underlying molecular mechanisms. While silencing HIF-1α in HepG2 human hepatoma cells did not alter the protein expression of NRF2, NRF2 knockdown markedly reduced the nuclear accumulation of HIF-1α without influencing its mRNA expression. In diethylnitrosamine-induced murine hepatocarcinogenesis, there was elevated NRF2 expression with concomitant upregulation of HIF-1α. NRF2 and HIF-1α co-localize and physically interact with each other. In addition, the interaction between NRF2 and HIF-1α was found in specimens obtained from HCC patients. In normoxia, HIF-1α undergoes hydroxylation by a specific HIF-prolyl hydroxylase domain protein (PHD), which facilitates ubiquitination and proteasomal degradation of HIF-1α. However, direct interaction with NRF2 hampers the PHD2-mediated hydroxylation and subsequent recruitment of von-Hippel-Linda for ubiquitination of HIF-1α. This results in the prolonged stabilization of HIF-1α, even in the presence of oxygen (pseudohypoxia), which may account for the HIF-1α -mediated aerobic glycolysis (Warburg effect).

O7: Aberrant BACH1/Nrf2 axis in Down Syndrome brain contributes to early onset Alzheimer-like neuropathology

Sara Pagnotta, Antonella Tramutola, Eugenio Barone, Fabio di Domenico and Marzia Perluigi

Department of Biochemical Sciences, Sapienza University of Rome

Several studies support the implication of aberrant redox phenotype in the brain of people with Down Syndrome (DS). Indeed, by mapping chromosome 21 several genes, such as SOD-1, BACH1, APP, CBR and S100B, are involved in the over-production of ROS in DS individuals. In particular, we investigated the role of BACH1 in the brain and its implication in the failure of the antioxidant response and autophagy system in human autoptic cases and in a mouse

model of DS (Ts2Cje). Our results revealed that BACH1 overexpression impairs the BACH1/NRF2 ratio in the nucleus as well as the induction of antioxidant response genes and a number of autophagic genes ultimately resulting in the accumulation of oxidative damage. Recent evidences on neurons and astrocytes isolated from Tg mice suggest that these processes act in concert to cause cell death via ferroptosis. Our findings suggest that BACH1 triplication in DS subjects modulate multiple downstream targets that altogether might contribute to accelerate the neurodegenerative process, ultimately resulting in early onset Alzheimer disease.

O8: Screening natural products for the identification of NRF2 activity modulators
Despoina D Gianniou1, Ioanna Kentroti1, Ioannis P. Trougakos1,2
1 Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece
2 GenCell-TSBiotech (https://gencell.gr/), Athens, Greece

Aging is a complex biological process 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, mitostatic and proteostatic modules is central to genome, mitochondrial and proteome stability. The antioxidant response system comprising 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 (among others) oxidative stress, thereby protecting against pathologies that relate to cellular biomolecules damage. We have shown the dose- and tissue-dependent activity of the NRF2/KEAP1 regulatory network during lifetime, as well as that NRF2 is part of a longevity promoting circuit that ensures functional wiring of proteostatic and mitostatic modules and consequently organismal survival during stress. We have also found a cytoprotective role of NRF2 in various age-related degenerative diseases, as well as that selected natural products can have a positive impact on cellular functionality, also via the activation of the NRF2 pathway, strongly supporting the notion that they can act as potential aging preventive agents and/or therapeutics for age-related disorders. Our rational and experimental platforms (both cell-free and cell-based, as well as at in vivo experimental models) for identifying modulators of NRF2 activity with anti-ageing and/or anti-age-related diseases (e.g., neurodegeneration, etc.) activity, along with some relevant recent findings, will be presented. Acknowledgments: IPT thanks NKUA SARG (C.S. 19067) for support.

O9: Identification of Novel Keap1 Inhibitors Using Machine Learning, Molecular Modeling and Molecular Dynamics Simulations. Kemal Yelekçi, Alp Eren Kazar, Ceren Cebeci
Kadir Has University, Faculty of Engineering and Basic Sciences, Department of Molecular Biology and Genetics, Fatih, Istanbul, Türkiye

The structure of human Keap1 Kelch domain (PDB: 4IFN) was retrieved from the protein data bank. The dataset of Keap1 inhibitors was downloaded from the CheMBL website (https://www.ebi.ac.uk/chembl/) with their respective IC_{50} values and SMILES codes. First, the compounds were desalted by the “SaltRemover” function of RDKit. The compounds

having null IC50 values were eliminated. Also, the compounds having duplicate IC50 values were removed as well. The threshold for IC_{50} values was determined to be 50 mM. The compounds with IC_{50} values equal to or lower than 50 µM were classified as" active", while those with the greater IC_{50} values were classified as "inactive". At the end, a set of suitable compounds was left for feature selection and model building. A molecular descriptors/features calculator called "PaDEL" was installed to identify specific descriptors such as hydrogen bond donor, hydrogen bond acceptor, molecular weight, Lipinski, SLogP value, Rotatable bond, and TopoPSA. Two-dimensional descriptors and fingerprints were calculated based on the SMILES of all compounds. The descriptors contain the computed features' specific topological and physicochemical properties by assigning a quantitative value for each feature.

The compounds were split into a training and test set for model building and evaluation. Feature selection was applied, and a refined set of molecular features was obtained using PaDEL as the descriptor program. Using the Matthew correlation coefficient, a training set, a test set, and a mean value have been determined for random forest model building. The most effective classification algorithm was generated for handling datasets requiring only a ligand SMILES string as input for rapid screening against the Keap1 target. Additionally, molecular docking studies and molecular dynamics simulations were used to analyze the most potent compounds' interactions with Keap1, thus evaluating the most stable ligand–Keap1 complexes. This novel framework aims to provide a fast, novel, and generalizable approach for predicting diverse compounds to be used as an inhibitor for Keap1. *Conflict of interest*: None. *Acknowledgments*: AEK thanks TÜBİTAK (PN: 2209) for support.

O10: Activation of Nrf2 Signaling and Inflammation Reduction by Dietary Broccoli Sprouts in Cafeteria Diet-Fed Mice

Marian V. Ivanochko, Maria M. Bayliak, Dmytro V. Gospodaryov, Volodymyr I. Lushchak
Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, 57 Shevchenko Str., Ivano-Frankivsk, 76018, Ukraine

Introduction. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key regulator of antioxidant defense, xenobiotic detoxification, and proinflammatory processes. Nrf2 activators, such as sulforaphane found in broccoli sprouts (BS), are being studied for their potential therapeutic roles in managing obesity and its associated metabolic complications. Materials and methods. This study examined the effects of dietary BS supplementation on the activities of Nrf2 target enzymes in the liver and cerebral cortex of eight-month-old C57BL/6J male mice reared on an obesogenic cafeteria diet (CD). Mice were fed for 16 weeks on one of four diets: 1) standard rodent feed (control), 2) standard feed with 5% of three-day-old BS, 3) CD, or 4) CD with 5% BS (CD+BS). Results. BS supplementation resulted to higher in-gel activities of glutathione S-transferases alpha and pi, and glutathione peroxidase in the murine liver compared to the control and mice fed CD. The activity of glutathione reductase was the highest in mice fed CD+BS. In the cerebral cortex, activities of glutathione reductase, glucose 6-phosphate dehydrogenase, and NAD(P)H-quinone oxidoreductase 1 were most elevated in the CD+BS group. Additionally, CD feeding significantly raised hepatic mRNA levels of genes CCL2 and IL6, markers of inflammation. In contrast, BS and CD+BS diets upregulated PPARGC1A, which encodes PGC-1α, a master

regulator of mitochondrial biogenesis and anti-inflammatory pathways downstream of Nrf2 signaling. Conclusions. These findings highlight the potential of BS supplementation to activate Nrf2-dependent antioxidant responses and attenuate obesity-induced inflammation in the murine liver and brain.

O11: Towards (marine) nutraceutical based ferroptosis intervention strategies to support aging healthcare

Wim Vanden Berghe

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Non-canonical Nrf2-dependent ferroptosis is a specific type of programmed cell death where a cell dies because its membrane is attacked by oxidative rust and disintegrates. In recent years, ferroptosis manifests across a spectrum of maladies with profound implications for human well-being. Numerous investigations substantiate that modulating ferroptosis, whether through inhibition or augmentation, plays a pivotal role in the etiology and control of numerous age-related afflictions, encompassing metabolic, cardiovascular, oncological, respiratory, neurological disorders and infectious diseases.

To better discriminate between biological and pathophysiological aging processes, by a dynamic interplay of metabolic, genetic, epigenetic, environmental, nutritional (diet) and microbiomal factors, we apply multi-omic ferroptosis signature approaches to define epigenetic age acceleration as a prognostic health-disease risk biomarker.

(Marine) plants possess primitive defense mechanisms, being in an early stage of evolution and lacking an immune system like ours. Instead of white blood cells, they rely on oxidative iron metabolic responses for defense. This ancient mechanism is also found in marine organisms such as seaweeds and algae, which lack immune systems and are packed with healthy antioxidants, minerals and vitamins to manipulate oxidative rusting ferroptosis systems to survive. We envision translational prospects of potential green-blue biotechnological applications of ferroptosis-targeted (marine) nutraceutical interventions to support aging healthcare.

O12: NRF2 signaling emerges as a key mediator in the molecular interplay between defective proteostasis and metabolic dysfunction in neurodegeneration

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Disruption of protein homeostasis (proteostasis) is increasingly recognized as a central factor in neurodegeneration, linking protein misfolding, oxidative stress, and cellular toxicity. Our research shows that disturbances in cellular stress response pathways, particularly the unfolded protein response (UPR), are closely associated with impairments in the NRF2-mediated antioxidant defense system. Concurrently, metabolic dysfunction correlates with proteostasis disruption and influences NRF2 signaling, together contributing to cognitive deficits. Using both in vitro and in vivo models, we investigated how metabolic stress impacts proteostasis and redox balance through the modulation of NRF2 activity. Furthermore, we demonstrate that pharmacological targeting of proteostasis or metabolically-driven stress responses can reduce oxidative damage and improve cellular resilience by restoring NRF2 function. These findings suggest that metabolic imbalance can

intensify proteostasis failure and redox dysregulation, promoting neurodegenerative changes and cognitive decline. NRF2 emerges as a critical effector in coordinating cellular damage and repair mechanisms. Targeting these interconnected pathways, particularly through the modulation of NRF2 and restoration of redox homeostasis, may offer promising therapeutic strategies for neurodegenerative disorders.

O13: Pharmacological Inhibition of Nrf2 as a Strategy to Overcome Therapy Resistance in Cancer

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Aberrant activation of the Nrf2 (nuclear factor erythroid 2–related factor 2) signaling pathway has emerged as a major mechanism by which cancer cells evade therapeutic stress. While Nrf2 plays a protective role in normal cells by regulating antioxidant and detoxification genes, its persistent upregulation in tumors—often due to mutations in KEAP1 or NFE2L2—drives drug resistance, metabolic adaptation, and immune escape. This presentation explores the therapeutic potential of pharmacologically inhibiting Nrf2 in cancers characterized by redox imbalance and resistance to standard treatments. I will outline current strategies for small-molecule inhibition of Nrf2, including direct inhibitors (e.g., ML385, PhcY), indirect Keap1-stabilizing agents (e.g., K67), and upstream modulators derived from natural products (e.g., apigenin, brusatol). Mechanistic insights, molecular targets, and efficacy data from preclinical studies will be highlighted, alongside challenges such as toxicity, off-target effects, and delivery barriers. Special attention will be given to the rationale for Nrf2 inhibition in non-small cell lung cancer, hepatocellular carcinoma, and breast cancer, where restoring therapy sensitivity through redox disruption has shown promising results. Finally, I will discuss emerging directions such as PROTACs, combination therapies with chemotherapy and immunotherapy, and biomarker-based patient stratification. Targeting the Nrf2 pathway represents a novel and underexplored approach to dismantling cancer's adaptive shield—offering new hope for tackling treatment-resistant malignancies.

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Sex differences play a key role in the development and severity of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and its progression to Metabolic Dysfunction-Associated Steatohepatitis (MASH), conditions linked to metabolic syndrome, obesity, and type 2 diabetes. The issue of sex-differences in the context of hepatic steatosis and NRF2-dependent proteostatic control is particularly worthy of investigation, as most preclinical studies have primarily focused on male models. This study aimed to investigate how sex influences the NRF2-mediated oxidative stress response, autophagy, unfolded protein response (UPR) signaling, and inflammatory markers in rats subjected to chronic metabolic challenges. Two dietary models were used over 12 weeks: a High-Fat and High-Fructose (HFHFr) diet to mimic MASLD, and a Choline-Deficient, L-Amino Acid-Defined (CDAA) diet to model MASH. The HFHFr diet primarily induced fatty liver and early-stage steatosis in females, whereas the CDAA diet led to steatohepatitis, fibrosis, and more advanced liver pathology in males. During the early stages of MASLD, males exhibited more effective NRF2-driven antioxidant defenses and autophagy-mediated proteostasis, while females showed reduced autophagic activity, potentially due to altered KEAP1-NRF2 regulation. In the advanced MASH model, despite similar NRF2 and KEAP1 transcript levels between sexes, CDAA-fed males displayed elevated inflammatory markers (e.g., TNF-α, F4/80), impaired autophagy, and an attenuated IRE1-XBP1 response. These findings highlight sex-specific differences in NRF2 signaling, autophagy, and ER stress responses during MASLD/MASH progression, underscoring the importance of sex-informed, NRF2-centered therapeutic strategies for chronic liver diseases.

O15: NRF2- AS A KEY REGULATOR IN INTESTINAL INFLAMMATION INDUCED BY SILVER NANOPARTICLES AND ITS MODULATION BY QUERCETIN

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Prolonged oral exposure of silver nanoparticles (AgNP) may result in significant accumulation in intestinal cells, potentially triggering inflammation, oxidative stress, and cytokine release. This study investigated the effects of polyvinylpyrrolidone (PVP)-coated AgNP of two distinct sizes (5 and 50 nm) on intestinal epithelial C2BBe1 cells, with a focus on nuclear factor erythroid 2-related factor 2 (NRF2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) expression, as well as the production of reactive nitrogen species. Additionally, the potential protective effect of quercetin, a flavonoid renowned for its antioxidant properties, was evaluated. Exposure to both sizes of PVP-AgNP resulted in a significant downregulation of NRF2 expression, as evidenced by decreased levels of p-NRF2. Both AgNP' sizes activated the NF-κB pathway, leading to increased levels of cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), and pro-inflammatory cytokines. Notably, activation mechanisms differed between the two sizes: the smaller nanoparticles (5 nm) activated NF-κB via the p65 pathway, whereas the larger nanoparticles (50 nm) triggered its activation through the IκBα-dependent pathway. Additionally, both sizes induced a significant upregulation of inducible nitric oxide synthase (iNOS) expression and nitric oxide (●NO) production. Quercetin exhibited protective effects, particularly in cells

exposed to 5 nm PVP-AgNP. It restored NRF2 expression, reduced iNOS levels, and significantly lowered ●NO production. Furthermore, quercetin decreased PGE2 and IL-8 production, highlighting its protective role in modulating inflammation and nitrosative stress. These findings suggest that targeting NRF2 pathways could offer a promising approach for preventing or treating inflammation-related disorders in the intestinal epithelium caused by nanoparticle exposure.

O16: Repurposing Omaveloxolone as a potential therapeutic approach for Early Age-Related Macular Degeneration

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Age-related macular degeneration (AMD) is characterized by progressive degeneration of the retinal pigment epithelium (RPE), contributing to photoreceptor loss and vision impairment. To model early AMD pathology, we established an in vitro system in which human RPE cells are challenged with porcine photoreceptor outer segments (POS), leading to lysosomal dysfunction and accumulation of autofluorescent granules (AFGs) that resemble lipofuscin deposits observed in vivo. These undigested materials are thought to drive oxidative stress and impair RPE viability. In parallel, we are validating an in vivo mouse model of AMD using a single intraperitoneal injection of sodium iodate ($NaIO_3$), which selectively targets RPE cells and induces oxidative stress. This model recapitulates key AMD features, including increased fundus autofluorescence, retinal thinning, and focal RPE cell loss followed by photoreceptor degeneration. NRF2 is a master regulator of antioxidant defense, inflammation, and autophagy. Given the central role of oxidative stress in AMD, we investigated the therapeutic potential of Omaveloxolone (OMV), an FDA-approved NRF2 activator, in our AMD models. The in vitro results demonstrate that OMV reduces AFG accumulation in POS-fed RPE cells and induces NRF2 protein expression within 2 hours of treatment, followed by upregulation of its downstream targets, HO-1 and NQO1. Preliminary in vivo data show that OMV prevents $NaIO_3$-induced retinal thinning and preserves RPE electrical activity. These findings support the repurposing of NRF2 activators such as OMV as a promising therapeutic strategy to preserve RPE function and slow AMD progression, particularly in early or intermediate stages where no approved treatments are currently available.

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O17: NRF2 induction coupled with cellular delivery of carbon monoxide for counteracting inflammatory conditions

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NRF2 is a fundamental stress responsive transcription factor that controls the expression of several antioxidant and inflammation-related genes, including heme oxygenase-1 (HO-1). The main function of HO-1 is to degrade heme to carbon monoxide (CO) and

biliverdin/bilirubin that are downstream effector molecules possessing protective and signaling properties, partially explaining the beneficial consequence of NRF2 activation. A variety of cellular pathways underlie the development but also in the rescuing of many chronic diseases. Therefore we hypothesized that creating hybrid compounds (HYCOs) consisting of CO-releasing molecules conjugated to NRF2 inducers could represent an advantageous approach to counteract conditions that are characterized by inflammation and oxidative stress. We synthesized and studied several HYCOs that were tested in cells and in vivo models with interesting results. Selected HYCOs, especially those containing Mn-based CO-releasing molecules, caused a significant increase in intracellular CO and augmented NRF2/HO-1 in different cell types. HYCOs reduced markers of inflammation in inflammatory cells challenged with LPS, while they decreased damage and myoglobin release in cardiomyocytes subjected to hypoxia-reoxygenation. Different HYCOs used in vivo modulated inflammation in models of LPS, psoriasis and multiple sclerosis, decreasing IL-8 production, erythema and scaling cores in psoriasis and the clinical manifestation of multiple sclerosis when given daily by oral gavage. Interestingly, the effect of these compounds was superior to that of NRF2 inducers or CO-releasing molecules alone.
Thus, developing dual activity molecules that activate NRF2 transcription and mimic HO-1-derived CO is a promising strategy for application in conditions where inflammation and oxidative stress are predominant features.

O18: Dissecting the protein interactome of wild type and lung cancer associated gain-of-function mutant forms of NRF2

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Gain-of-function (GOF) mutations in NRF2 are frequently observed in squamous cell lung cancer and are associated with poor patient prognosis due to resulting NRF2 hyperactivation. These mutations are typically localized in two hotspot regions within the KEAP1-interacting DLG and ETGE motifs of NRF2, leading to impaired ubiquitination, decreased degradation, and consequent stabilization of the protein. Although NRF2 is recognized as a key oncogenic transcription factor, its protein interaction network remains poorly characterized, largely due to the inherent challenges of transcription factor proteomics. In this study, we investigated the interactome of wild-type NRF2 and two GOF mutants, D29N and E79Q, located within the DLG and ETGE domains, respectively. We employed proximity-dependent biotin identification (BioID) combined with mass spectrometry to map protein-protein interactions. Additionally, we integrated chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq) to assess changes in chromatin binding and gene expression. Proteomic analysis revealed that GOF mutants of NRF2 translocate to the nucleus and associate with chromatin more robustly than wild-type NRF2. BioID profiling identified several known NRF2 interactors, such as KEAP1, MAFG, and PGAM5, alongside a set of novel binding partners. Notably, the GOF mutations of NRF2 significantly enhanced interactions with chromatin-associated proteins, including components of the SWI/SNF chromatin remodeling complex, as well as various transcriptional coactivators and coregulators. These altered interactions were reflected in widespread changes in NRF2 chromatin occupancy and downstream target gene

expression. We propose that oncogenic NRF2 mutations promote cancer progression by enhancing its engagement with chromatin-modifying proteins, thereby reshaping the epigenetic landscape to drive oncogenic transcriptional programs.

O19: Dual Modulation of NRF2 by KEAP1 and GSK3: A Shared Therapeutic Axis in Alzheimer's Disease and MASH

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Glycogen synthase kinase-3 (GSK3) is a central kinase implicated in diverse pathologies, ranging from Alzheimer's disease (AD) to non-alcoholic steatohepatitis (NASH/MASH). In AD, hyperactivation of GSK3 drives tau hyperphosphorylation and neurofibrillary tangle formation, while in NASH, GSK3 contributes to hepatocellular injury, inflammation, and fibrosis. A common downstream target of GSK3 is the transcription factor NRF2, a master regulator of the antioxidant response. NRF2 stability is controlled by two complementary degradation systems: the canonical KEAP1–Cul3 axis and the alternative GSK3–β-TrCP pathway. In early disease states, oxidative stress disables KEAP1, permitting NRF2 activation as an adaptive response. However, in advanced disease, hyperactive GSK3 phosphorylates NRF2, promoting β-TrCP-mediated ubiquitination and proteasomal degradation, thereby silencing cytoprotective transcriptional programs. This mechanistic convergence suggests that dual inhibition of KEAP1 and GSK3 could restore NRF2 function across disease stages. 6-(Methylsulfinyl)hexyl isothiocyanate (6-MSITC, hexarafane), a naturally occurring isothiocyanate, exerts such dual activity: it disrupts KEAP1-dependent repression while simultaneously limiting GSK3-mediated NRF2 degradation. Thus, 6-MSITC provides a conceptual therapeutic bridge between neurodegeneration and metabolic liver disease, offering stage-independent reactivation of NRF2 signaling. Exploring this dual regulatory axis may open new avenues for interventions that simultaneously target redox imbalance, inflammation, and maladaptive protein phosphorylation across seemingly distinct chronic disorders.

O20: Targeting NF-κB–Mediated Neuroinflammation to Recalibrate NRF2 Signaling and Mitigate Oxidative and Ferroptotic Stress in Parkinson's Disease

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Parkinson's disease (PD) is marked by dopaminergic neurodegeneration, chronic neuroinflammation, oxidative stress, and disrupted proteostasis. NF-κB is a central transcription factor driving the pro-inflammatory cascade in PD, and its sustained activation perpetuates glial reactivity, inflammasome assembly, and cytokine release, further

aggravating neuronal injury. Here, we show that pharmacological inhibition of serum- and glucocorticoid-regulated kinase 1 (SGK1) with the novel brain-penetrant compound EMM-3.20 effectively suppresses NF-κB signaling in an α-synuclein overexpression mouse model of PD. This suppression normalizes the expression of NLRP3 inflammasome components, reduces astrogliosis and microgliosis, and attenuates pro-inflammatory cytokine production. Importantly, dampening NF-κB–driven neuroinflammation also modulates the NRF2 antioxidant pathway, as evidenced by the reversal of α-synuclein–induced upregulation of NRF2 and its target genes (Hmox1, Nqo1, Gpx1), indicating reduced oxidative stress demand. NRF2-linked processes, including regulation of ferroptosis, lipid peroxidation, and mitochondrial homeostasis, were also favorably impacted, suggesting a broad restoration of redox balance. Together, these findings reveal that targeting NF-κB–mediated inflammatory signaling upstream can indirectly recalibrate NRF2 activity and its protective transcriptional program, thereby mitigating oxidative, ferroptotic, and mitochondrial stress. This NF-κB–NRF2 axis modulation via SGK1 inhibition emerges as a promising disease-modifying strategy for PD and related neurodegenerative disorders with intertwined inflammatory and oxidative pathology.

O21: Nrf2 Signaling as a Mediator/ Enhancer of Statin- and SGLT2 Inhibitor Benefits in Metabolic Disease

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The transcription factor Nrf2 integrates cytoprotective functions with regulation of key metabolic pathways, including gluconeogenesis and lipogenesis. While its activation protects against hyperglycemia, fatty liver, and insulin resistance, its modulation by frontline metabolic disease drugs is poorly characterized.

We examined Nrf2 pathway activation by two major drug classes—statins and SGLT2 inhibitors—widely prescribed for dyslipidemia and diabetes.

In male Wistar rats (n=5), simvastatin (120 mg/kg/day, 5 days, gavage) significantly increased hepatic Nrf2 transcriptional activity and upregulated target gene expression (EMSA). This was confirmed in primary hepatocytes and the ST2 cell line, revealing cholesterol-independent but mevalonate-dependent mechanisms.

In wild-type C57Bl/6 male mice, chronic canagliflozin treatment (200 mg/kg chow) was assessed in two settings: a longevity study (n=175; 83 control, 92 canagliflozin) and a 3-month exposure study (n=25). Canagliflozin extended median survival by 5 weeks (112.5 vs 107.5 weeks, $p<0.05$). Transcriptomic analysis in the short-term cohort revealed robust Nrf2 pathway activation in liver and adipose tissue. These findings suggest that Nrf2 signaling may mediate—and potentially amplify—the beneficial effects of statins and SGLT2 inhibitors. Targeting Nrf2 alongside these treatments could yield additive or synergistic benefits. Ongoing work in Nrf2 knockdown models will clarify its causal role in drug action. (In loving memory of my mentor, Thomas W. Kensler.

O22: Nrf2 in MASH; Potential Role of Lipid Droplet Maturation

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Metabolic dysfunction-associated steatotic liver disease (MASLD) has emerged as the most widespread chronic liver condition, starting with simple hepatic steatosis that can progress to metabolic dysfunction-associated steatohepatitis (MASH), and hepatic fibrosis. Lipid droplets (LDs) are ubiquitous organelles not only specialized in lipid storage, but also participate in inflammatory and metabolic dieases. Regarding the crucial role of LD accumulation in MASH development, understanding signaling mediators regulating LD biogenesis may give alternate views on disease pathogenesis and treatment approaches. Nrf2 is a major transcription factor that plays a pivotal role in regulating the response against oxidative stress and inflammation. Although it is known to involve in MASH development, how Nrf2 signaling is affected in LD biogenesis is not yet clear.

In our study, we established an in vivo MASH model in mice fed cholesterol-, palmitate-, trans fat-rich diet, and reduced LD biogenesis by specifically inhibiting the hepatic Seipin expression using metabolically stable siRNAs that are conjugated to a synthetic N-acetylgalactosamine (GalNAc). Focusing on alterations in disease development, we tested Nrf2 status and LD biogenesis in our in vivo disease model. Our initial finding point out both Nrf2 and Seipin protein as an emerging parameters to involve in the transition from steatosis to MASH. Further studies include the role of hepatic Seipin inhibition in MASH development together with Nrf2 status. Overall, our findings could represent a novel interaction between LD biogenesis and Nrf2 that wll contribute to treatment approaches against MASH. Supported by The Scientific And Technological Research Council Of Turkey (TUBITAK) 223S770.

O23: The thyroid as a sentinel and target organ in Keap1/Nrf2 pathway activation: lessons from animal models and human studies, and implications for Nrf2-targeted therapies.

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Hormonal systems interact with stress signaling pathways to maintain homeostasis throughout life. In vertebrates, the thyroid gland is central to metabolic regulation, controlling oxygen consumption and resting energy expenditure via secretion of thyroxine (T4) and triiodothyronine (T3). Thyroid hormone synthesis requires hydrogen peroxide dependent oxidation reactions that iodinate thyroglobulin (TG), the hormone precursor. Evidence from mice and humans demonstrates a tight link between the Keap1/Nrf2 antioxidant pathway and thyroid physiology, including direct Nrf2 mediated transcriptional control of TG via two AREs. Keap1 hypomorphic mice, with constitutively active Nrf2, develop goiter and mild biochemical hypothyroidism but show no extra thyroidal defects under normal conditions. Similarly, a heterozygous KEAP1 loss of function mutation in a Japanese family segregated with goiter over five generations without other phenotypes. These findings identify the thyroid as highly sensitive to genetic Nrf2 activation and raise potential clinical concerns for Nrf2 modulating drugs. Pharmacological Nrf2 activation might induce goiter, while inhibition could enhance oxidative stress sensitivity, favoring

autoimmune thyroid disease -- a possibility supported by NRF2 promoter polymorphisms linked to thyroid autoimmunity. Future work should determine whether post natal Nrf2 activation alone causes goiter, and whether dietary or environmental factors modulate Keap1/Nrf2 dependent thyroid phenotypes. Preclinical testing of clinically used Nrf2 modulators and monitoring patients for thyroid effects are warranted. At this closing meeting of the BenBedPhar COST Action, we present completed and preliminary studies on Keap1 hypomorphic mice, discuss goiter pathogenesis, and outline future collaborative research arising from the Action.

O24: Nrf2 is a transcriptional regulator of CD5L

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CD5 antigen-like (CD5L), also known as apoptosis inhibitor expressed by macrophages (AIM), is a protein, which is secreted by macrophages and participates in multiple biological processes, including inflammation and infection. CD5L can be transcriptionally upregulated by liver X receptor-a (LXRa)/retinoid X receptor (RXR) complex, MafB, sterol regulatory element-binding protein 1a (SREBP-1a), and signal transducer and activator of transcription 3 (STAT3). A comparative analysis of the proteomes of bone marrow-derived macrophages (BMDMs) from wild-type (WT), Nrf2-knockout (Nrf2-KO) and Keap1-knockdown (Keap1-KD) mice identified CD5L among the differentially abundant proteins, where in comparison with WT BMDMs, the copy number of CD5L was higher in Keap1-KD and lower in their Nrf2-KO counterparts. A follow-up study showed that genetic or pharmacological activation of Nrf2 increased the mRNA levels of CD5L, similar to the classical Nrf2 target NAD(P)H: quinone oxidoreductase 1 (NQO1). Conversely, depletion of Nrf2 resulted in a decrease in CD5L mRNA levels. Moreover, the potency of small-molecule Nrf2 activators with distinct mechanisms of action and potencies, correlated with the extent of CD5L expression, both at the mRNA and the secreted protein levels. The induction of CD5L by the Nrf2 activator omaveloxolone was also observed in primary human macrophages. CRISPR/Cas9 gene editing-mediated deletion of two sequences resembling the antioxidant response element (ARE) Nrf2-binding motif, located 20,000 bp upstream of the Cd5l promoter, led to a profound decrease in CD5L mRNA levels. Together, these findings identify Nrf2 as a transcriptional regulator of CD5L.

O25: Vascular effects of dimethyl fumarate in male hereditary hypertriglyceridemic rats exposed to social stress

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Hypertriglyceridemia and chronic social stress are recognized risk factors for cardiometabolic disorders. We investigated vascular function in adult male normotensive male Wistar-Kyoto (WKY), borderline hypertensive (BHR), and hypertriglyceridemic (HTG) rats. The effects of chronic crowding stress and the NRF2 activator dimethyl fumarate (DMF; 20 mg/kg/day, p.o., 4 weeks) on vascular function in HTG rats were also examined. BHR and HTG rats exhibited elevated blood pressure and augmented noradrenaline- and K^+-induced

contractions in femoral (FA) and mesenteric (MA) arteries vs. WKY. In HTG, but not BHR, acetylcholine (ACh)-induced relaxation was impaired in both arteries, while plasma triglycerides, atherogenic index, and glycemia were increased. In HTG, social stress significantly potentiated maximal K^+- and serotonin-induced FA constrictions. MA responses were unaltered. DMF reduced K^+- and noradrenaline-induced MA, but not FA, contractions. In addition, stress diminished ACh-induced relaxation in the FA and DMF failed to restore it. In MA, stress and DMF interacted significantly: the lowest ACh-induced relaxation occurred in DMF-treated HTG rats exposed to stress. DMF also elevated expression of Nfe2l2, Hmox1, and Ppara genes in the heart. In conclusion, hypertriglyceridemia with prehypertension exerted more detrimental vascular effects than prehypertension alone. While DMF selectively attenuated K^+- and noradrenaline-induced contractions in HTG rats, DMF did not improve, and, under stress conditions, it even exacerbated endothelial dysfunction in the MA. The results thus indicate caution when DMF is used due to its effects on vascular function, especially under conditions of metabolic dysregulation or chronic stress. Supported by APVV-22-0296 and MVTS-CA20121.

ABSTRACTS TO POSTERS

P1: Nrf2 modulates immune responses in sepsis by regulating PANoptosis patterns in PBMCs

Qian Zhang

Nrf2 is an important immune mediator linking oxidative stress and inflammatory signaling pathways as well as cell survival. Therefore, in this study, we aimed to check the role of Nrf2 in immune responses against sepsis via regulating PANoptosis in PBMCs

P2: Design and validation of a SIMOA platform for the measurement of Nrf2 levels

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Nrf2 is a transcription factor that plays a key role in protecting cells from oxidative stress through intracellular signaling pathways. Disruption of the Nrf2 pathway is linked to the development of inflammatory and chronic diseases such as diabetes, atherosclerosis, and neurodegenerative disorders. Accurate measurement of Nrf2 levels and activity is valuable for both research and clinical applications, including diagnostics, drug response monitoring, and disease progression analysis.

This study focuses on developing a highly sensitive assay for quantifying Nrf2 using Single Molecule Array (SIMOA) technology. SIMOA, also known as digital ELISA, is a bead-based platform that allows ultra-sensitive detection of low-abundance proteins by capturing targets on antibody-coated paramagnetic beads.

To build the SIMOA assay, two anti-Nrf2 antibodies recognizing distinct epitopes were required. Since the 3D structure of Nrf2 is unresolved, we first created a computational model to predict accessible epitopes. A panel of commercial antibodies was evaluated

based on these predictions. Surface Plasmon Resonance (SPR) analysis identified high-affinity antibodies with dissociation constants in the picomolar range. Competitive binding assays via SPR and ELISA confirmed the selected antibodies bind non-competitively to distant epitopes.
In the final assay design, the capture antibody was immobilized on magnetic beads, while the detection antibody was biotinylated. The developed SIMOA kit was tested using cell lysates, monocytes from Alzheimer's disease patients, and plasma samples. This is the first report of a SIMOA-based assay for Nrf2, enabling highly sensitive and specific quantification of Nrf2 in biological samples.

P3: Heme oxygenase-1 does not affect formation of stress granules in iPS cells

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Heme oxygenase-1 (HO-1) is an inducible enzyme involved in cellular defense, degrading free heme into biliverdin, carbon monoxide, and ferrous iron. Beyond this, HO-1 participates in stress-response pathways, including oxidative and proteotoxic stress. Stress granules (SGs) are transient cytoplasmic assemblies of untranslated mRNPs, translation initiation factors, and RNA-binding proteins that help modulate mRNA translation during stress.This study examined whether HO-1 influences SG formation in murine induced pluripotent stem (iPS) cells exposed to sodium arsenite (oxidative stress) or MG-132 (proteotoxic stress). Using immunocytochemistry and fluorescence microscopy, we assessed SG assembly alongside HO-1 expression and localization. Our results showed that HO-1, whether in the nucleus or cytoplasm, did not significantly affect SG number, size, or distribution under either stress condition. Thus, HO-1 does not directly regulate SG dynamics in these contexts. Notably, we found that overexpression of unrelated proteins increased SG formation following MG-132 treatment. This suggests that the accumulation of excess proteins, rather than specific regulatory proteins like HO-1, drives SG formation under proteotoxic stress. In conclusion, while HO-1 is vital for antioxidant defense, it does not appear to influence SG formation during oxidative or proteotoxic stress in iPS cells. Our findings emphasize the role of protein homeostasis—rather than specific signaling modulators—in shaping SG responses under proteasome inhibition, offering new insights into the cellular mechanisms of stress adaptation.

P4: The transcription factor GATA4 is expressed in liver progenitor cells and confers protection against epithelial-mesenchymal transition

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Activation of liver progenitor cells (LPCs) during chronic liver diseases is a repair regenerative response. The transcription factor GATA4 is essential for liver development.

Herein, we identified GATA expression in LPCs and investigated its role in epithelial-to-mesenchymal transition (EMT) induced by TGF-β and hypoxia. GATA4 expression was assessed in LPCs exposed to TGF-β or hypoxia (1% O2) and effects of its overexpression/silencing were investigated. Mice were transplanted wild-type or GATA4 overexpressing LPCs and injected CCl4. Fibrotic liver injury was analyzed. Expansion of the LPC niche was characterized in mice exposed to intermittent hypoxia (IH). GATA4 expression was determined in humans with primary biliary or primary sclerosing cholangitis (PBC and PSC). TGF-β decreased GATA4 in LPCs concurrently with EMT induction and a decrease of the LPC marker A6, revealing a loss of LPC identity. GATA4 overexpression protected against TGF-β-mediated EMT whereas GATA4 silencing markedly reduced LPC viability. Mice transplanted with GATA4 overexpressing LPCs were partly protected against CCl4-induced fibrosis. LPCs cells exposed to 1% O2 also showed GATA4 downregulation along with loss of A6 and EMT induction. Molecularly, HIF2α up-regulation concurred with elevation of nuclear NRF2, and its targets HO-1 and NQO1, pointing to an anti-oxidant compensatory response. Again, GATA4 overexpression attenuated hypoxia-induced loss of LPC identity. In livers from mice subjected to IH abundant A6-/GATA4-/αSMA+ niches were observed without features of HSCs, suggesting presence of de-differentiated LPCs. Expression levels of GATA4 in human liver samples was decreased in PSC patients. In conclusion, we uncovered a new role of this transcription factor in the liver by preserving LPC cell fate under EMT. Therefore, modulating GATA4 levels LPCs might offer therapeutic opportunities.

P5: Kino2omics integration platform bridging the Genotype and Phenotype gap in precision medicine

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Besides protein translation, phosphorylation is a reversible modification playing a crucial role in controlling the functional state of proteins in health and disease. Profiling protein kinase activity has become a critical component in molecular clinical biology, precision medicine, and constitutes a promising tool for drug discovery. However, current strategies can only monitor single kinases or require complex procedures and infrastructure, making their assessment challenging. The Kin2omics platform performs parallel measurement(s) of kinase activities by recording phosphorylation changes in real time, revealing a more realistic view of the cellular signaling states and molecular mechanisms operating in disease and drugs mode of action (https://www.kinases-epigenetics-ppes.com/) We use the Pamstation-12 (PamGene NVA, Netherlands), a fully automated instrument designed to process peptide microarrays (pamchips). This technology allows simultaneous measurement of multiple kinase activities (either activation or inhibition) through highly sensitive monitoring of the phosphorylation dynamics of 144 peptide substrates by Serine/ Threonine or 196 for Tyrosine kinases, present in (clinical) biosamples of interest (cells, tissues, organoids, ipscs, patients biopts). The team gained unique expertise in the profiling of kinase activity in a wide range of cancer cell lines (glioblastoma, pancreatic cancer, neuroblastoma, multiple myeloma, T-cell acute lymphoblastic leukaemia, breast cancer), cells and patient-derived xenografts, patient-derived organoids, mouse, and human tissue

samples (brain and colon), and patients' liquid biopsies. Moreover, the platform allows the "pharmacology on-chip" evaluation of kinase inhibitors that, together with the new systems biology core integrating kinome data with other omics techniques (e.g. transcriptome, phospho-proteome and epigenome data), will narrow the bridge between disease and drug discovery strategies. Our technology can be applied in a wide spectrum of fundamental, translational, diagnostic, and clinical research: a) Find novel targets, determine activities of kinase inhibitor drugs or novel food ingredients, and elucidate their mechanisms of action, b) to determine the mode of action of pharmacological compounds, c) to perform target interaction/ engagement studies, d) for Enzymatic characterization of novel or mutated kinases. e) for the identification of substrates for novel, mutated or post-translationally modified kinases, f) for prognosis and resistance Biomarker discovery and g) or evaluation of pharmacological drug dosage or combination therapy. We offer the service to academic research groups, clinical research labs as well as, drug screening, pharma, and biotech companies.

P6: Structure-Based Discovery and Optimization of KEAP1–NRF2 Inhibitors

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The KEAP1–NRF2 interaction constitutes a critical regulatory axis in maintaining redox homeostasis. While transient activation of NRF2 confers cytoprotective effects under oxidative stress, its sustained upregulation in malignant cells promotes tumor progression, therapeutic resistance, and adverse clinical outcomes. Accordingly, pharmacological disruption of the KEAP1–NRF2 protein–protein interaction has emerged as a promising strategy in anticancer drug development. In this study, we implemented a structure-based virtual screening protocol to explore a curated FDA-approved drug library, aiming to identify novel small-molecule inhibitors targeting the Kelch domain of KEAP1. Molecular docking simulations were carried out using Glide from Schrödinger Maestro, and the top-ranked ligands—based on binding affinity and pose quality—were subsequently subjected to 100 ns molecular dynamics simulations via Desmond to assess the stability of their interactions at the atomic level. Lead candidates exhibiting stable binding modes and robust interaction profiles were further optimized through rational derivative design to enhance target affinity and pharmacophoric complementarity. These refined molecules were evaluated using the MetaCore platform's anticancer prediction module to estimate their therapeutic potential across diverse oncological pathways and cellular contexts. This integrative in silico strategy facilitated the identification of KEAP1–NRF2 inhibitors with favorable drug-like properties and predicted antitumor activity, providing a strong foundation for future experimental validation. These findings underscore the translational relevance of targeting the KEAP1–NRF2 interface in cancer therapy and highlight the utility of computational pipelines in modern drug discovery.

P7: Update on Therapeutic Strategies Targeting Nrf2 Pathway Modulation in Kidney Diseases

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Nrf2 is a promising target to counteract the progression of both acute (AKI) and chronic kidney diseases (CKD). Although Keap1 inhibitors such as bardoxolone methyl showed early efficacy in Phase II trials for diabetic nephropathy, their clinical use has been limited due to cardiovascular adverse events and increased proteinuria. In contrast, two potent Nrf2 activators, omaveloxolone, approved for Friedreich's ataxia, and dimethyl fumarates (DMF), used in multiple sclerosis and psoriasis, have shown favorable safety profiles and are being explored for renal indications. While mild adverse effects have been reported, no severe cardio renal events have been observed. Alternatively, GSK3β inhibitors stabilize Nrf2 via a Keap1-independent mechanism. Compounds such as TDZD-8, SB216763, and low-dose lithium have improved renal outcomes in preclinical models of AKI and CKD. Our group has demonstrated that DJ-1-derived GSK3β inhibitors exert anti-inflammatory and anti-fibrotic effects in models of kidney injury. In parallel, Bach1 inhibitors are emerging as novel Nrf2 modulators. By relieving repression of HMOX1 and NQO1, they enhance cytoprotective responses. HPP-971 has shown good tolerability and renal efficacy. Our group observed trends toward improved renal function and reduced inflammation, although further validation is needed. These indirect Nrf2-targeting strategies may offer a safer therapeutic profile. However, the apparent safety of omaveloxolone and DMF compared to bardoxolone raises important mechanistic questions: What underlies their differential toxicity? Could they represent safer, more effective options for kidney disease? This presentation will address these questions and provide an updated overview of Nrf2 modulation as a therapeutic strategy in renal pathologies.

P8: Development of Bile Acid-Based Compounds as Dual Nrf2 Activators and FXR Modulators

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Targeting both redox balance and bile acid signaling presents a promising strategy for treating metabolic and inflammatory liver diseases. The nuclear factor erythroid 2–related factor 2 (Nrf2) and farnesoid X receptor (FXR) pathways play key roles in regulating oxidative stress and bile acid metabolism, respectively—two interlinked processes involved in hepatic inflammation and disease progression. In this study, we developed novel bile acid derivatives designed to act as dual modulators: Nrf2 activators and FXR antagonists. Chenodeoxycholic and cholic acid were used as starting scaffolds due to their structural compatibility with bile acid receptors. Key synthetic modifications included introduction of an enone moiety in the A ring, enabling covalent interaction with Keap1 and activation of Nrf2 signaling. Additionally, a C7-ethylidene group, introduced via regioselective Wittig reaction, served as a pharmacophore for FXR antagonism, inspired by known antagonists like 7-ethylidene-lithocholic acid (7-ELCA). These dual-acting compounds aim to promote cytoprotective gene expression while modulating bile acid signaling, offering potential benefits in diseases marked by oxidative stress and disrupted bile acid homeostasis. This approach provides a rational platform for the development of multifunctional therapeutic candidates targeting complex liver pathologies.

P9: Oxidative activity of cancer-selective carbon dots in vitro under physiological oxygen levels

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Cancer is a devastating disease characterized by limited treatment options and a low survival rate. Current therapies are often associated with significant drawbacks, including severe side effects and the development of chemoresistance, which lead to suboptimal treatment outcomes and reduced quality of life. Nanomedicine has emerged as a promising approach, leveraging nanoscale tools for precise cancer targeting. Among these, carbon dots (CDs) have gained significant attention due to their small size, aqueous solubility, intrinsic photoluminescence, and high biocompatibility. (Bartkowski M et al. Chemistry – A European Journal 2024). Our group has recently developed a method to obtain fluorescent CDs from spent coffee grounds (Zhou Y et al. Nanoscale 2025). Our CDs exhibited a small size (≈ 2 nm) and efficient cellular uptake in vitro. Notably, they showed selective toxicity, exerting antiproliferative effects the triple-negative breast cancer CAL-51 cell line by increased reactive oxygen species (ROS) production without harming healthy breast, kidney, or liver cells. Additionally, the accumulation of our CDs in cancer cells was not hindered by drug efflux pumps P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), suggesting their potential to overcome multidrug resistance in therapeutic applications. These promising results encourage us to further explore their activity and oxidative stress-based mechanism of action in a more extended panel of cancer cell lines and primary cells. However, to accurately assess their potential for clinical translation, it is crucial to consider physio-pathological oxygen levels during in vitro testing. Therefore, evaluation of the cytotoxicity of our CDs in the sensitive CAL-51 cell line and non-sensitive ovarian carcinoma SKOV-3 cell line, as well as in primary cells, at both physiological (5kPa) and conventional (18kPa) oxygen tension conditions is critical. Total and mitochondrial ROS generation, calcium dynamics, and oxidative stress scavenging enzyme activity are also assessed. These findings allow us to validate the effects and mechanism of action of our CDs in cancer and health cells, providing insights into their potential for clinical applications. Supported by Research Ireland and Breakthrough Cancer Research, Ireland.

P10: NRF2 isoform 2 reveals additional level of NRF2 regulation

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The NRF2 gene (NFE2L2) is expressed from two promoters, P1 and P2, generating transcripts differing only in their first exons. The P1 promoter produces canonical, full-

length NRF2 isoform 1, while the P2 promoter generates transcripts giving rise to isoform 2, which is 16 amino acids (~1.6 kDa) shorter at the N-terminus. Both P1 and P2 transcripts are expressed in various human tissues, as well as in other vertebrates, but the reason for the existence of two NFE2L2 promoters is unknown. With siRNA targeting specifically NFE2L2 P1 transcripts, we identified NRF2 isoform 2 in human cells and demonstrated that it regulates heme oxygenase 1 expression. We validated this result in canine cells, which express only the truncated NRF2 isoform, corresponding to human isoform 2. We also found that isoform 2, like isoform 1, is highly unstable. Polysome profiling revealed distinct modes of translational regulation between P1 and P2 transcripts. P1 transcripts are exclusively associated with actively translating polysomes, consistent with their efficient translation. In contrast, P2 transcripts are distributed between monosomes and polysomes, indicating that they are subject to translational regulation. These results suggest that while canonical NRF2 isoform 1 is regulated solely on the level of degradation, the NRF2 isoform 2 can be regulated at both translation and degradation levels. Notably, exon 1 of the P2 transcripts contains evolutionarily conserved sequence elements with potential regulatory activity, which may underlie this additional layer of control.

P11: Exploring the Role of DMF-driven NRF2 Activation in Lysosomal Function and Autophagy in RPE Cells

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Autophagy impairment and lysosomal dysfunction in retinal pigment epithelial (RPE) cells are central pathological features of age-related macular degeneration (AMD), contributing to the accumulation of autofluorescent undegraded materials such as lipofuscin.

The therapeutic potential of dimethyl fumarate (DMF), a clinically approved activator of the nuclear factor erythroid 2-related factor 2 (NRF2) pathway, in modulating autophagy, lysosomal function, and lipofuscin clearance in human RPE cells is being explored.

Our results show that DMF rapidly activates NRF2 signalling, leading to enhanced nuclear translocation and transcriptional upregulation of cytoprotective target genes, collectively contributing to increased autophagic flux and reduced oxidative stress. This work reports for the first time that photoreceptor outer segments (POS) phagocytosis is sufficient to trigger NRF2 activation, suggesting an intrinsic feedback mechanism, driven by phagocytic load that induces antioxidant and degradative stress responses to maintain cellular homeostasis. Furthermore, pharmacological induction of NRF2 by DMF significantly diminishes the accumulation of lipofuscin-like autofluorescent granules (AFG), a surrogate marker for impaired lysosomal degradation, indicating improved lysosomal clearance capacity. Importantly, both genetic silencing and pharmacological inhibition of autophagy abolish the beneficial effects of DMF on AFG reduction, underscoring the essential role of autophagic pathways in NRF2-mediated POS degradation. Our results provide mechanistic support for NRF2 activation, via DMF, as a promising therapeutic approach to restore RPE homeostasis, mitigate lipofuscin accumulation, and potentially slow the progression of retinal degeneration in AMD and related disorders.

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P12: NetRF2: network-based analysis of tissue- and cell-specific targets and interactions of NRF2

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NRF2, a transcription factor with a centralized role in redox biology, has been studied for its role in metabolism, cellular defense, and cancer. However, tissue- and cell-specific expression patterns of its targets and interactions and their role in human diseases are not well defined. We have developed and used NetRF2, a Shiny web application, to present these NRF2-relevant Human Protein Atlas (HPA) expression data in the form of mono- and/or multi-partite networks. With the integration of Human Protein Atlas (HPA) expression data from normal tissues and associated cells as well as cancer cells we were able to generate annotated networks of NRF2 targets and interactions for visualization and further analysis. Moreover, the users of NetRF2 can also further annotate these networks using their own list of genes along with log transformed fold changes (logFC) in the selected tissue/cells or cancers. We have tested NetRF2 using an RNA-seq dataset from NCBI GEO (GSE147304) on non-alcoholic steatohepatitis (NASH) and identified liver specific cell and tumor networks for NRF2 targets and interactions. By integrating gene and protein expression profiles with variant-level information, the app offers a cohesive framework to explore NRF2-related mechanisms across disease contexts.

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P13: Combining stress response augmentation and reductive stress relief through mild hypothermia

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Ischemia–reperfusion injury (IRI) remains a major challenge in hypoxia-related disorders. While cooling has long been linked to metabolic suppression, recent work shows that mild hypothermia (32 °C) also triggers active cytoprotective programs. Using in vitro models of hypoxia and reoxygenation, we investigated how temperature influences the balance between metabolic inhibition and stress tolerance. Mild hypothermia emerged as optimal, simultaneously reducing hypoxia-induced lactate:pyruvate elevation, preserving ATP/ADP ratios, and improving mitochondrial recovery. Importantly, 32 °C cooling activated NRF2-dependent antioxidant pathways, increasing thioredoxin- and glutathione-related gene expression and total glutathione, while avoiding oxidative overload. This temperature also preserved hypoxia response element activity, suggesting selective HIF pathway engagement without inducing deleterious downstream hypoxic responses. These results indicate that the benefits of mild hypothermia extend beyond metabolic slowing to include

NRF2-driven reinforcement of redox homeostasis and mitigation of reductive stress, offering a mechanistic basis for targeted therapeutic strategies in IRI.
Funding: This research was supported by Estonian Research Council PUT1077, PUT120, PRG685, IUT20-42, PSG959; ERDF2014-2020.4.01.15-0012 and Baltic Research Programme of the EA EEZ/BPP/VIAA/2021/8.

P14: NRF2 Transcription Factor: Sculpting Synaptic Lipids for Brain Health.
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Synaptic loss is a key factor in the cognitive decline observed during aging and in neurodegenerative diseases such as dementia, where synaptopathy plays a central role in hippocampal dysfunction. In this study, we investigated the role of NRF2, a master regulator of cellular homeostasis, in maintaining synaptic integrity. We assessed synaptic contacts both in vitro and in vivo and found that NRF2 deficiency leads to a significant reduction in vGLUT1 levels, accompanied by a decrease in the number of synaptic contacts. Because synapses are subject to highly dynamic membrane remodeling processes, we analyzed the lipid composition of hippocampi and synaptosomes from NRF2-deficient and wild-type mouse littermates. Our results revealed an accumulation of ether-linked phospholipids in NRF2-deficient mice. When primary neuronal and organotypic cultures were exposed to an ether-lipid precursor, synaptic density decreased. By contrast, the NRF2 activator 6-MSITC prevented synaptic loss. Although ether lipids are abundant components of neuronal membranes, their specific role in synaptic function and in age-related loss of homeostatic balance remains poorly understood. This study is the first to demonstrate that NRF2 plays an essential role in preserving synaptic homeostasis through lipid metabolism, suggesting its relevance in the context of aging and neurodegenerative diseases.

P15: NRF2 activators for treatment of neurodegeneration with brain iron accumulation
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Neurodegeneration with brain iron accumulation (NBIA) is a set of hereditary neurological disorders. Clinically, NBIA disorders share symptoms of iron accumulation in basal ganglia and other brain structures in childhood, resulting in early Parkinsonism and other complications. Genetically, invalidating mutations in genes with various and often unknown functions are causative for NBIA. On a cellular level, NBIA associated changes involve mitochondrial dysfunction, oxidative stress, iron accumulation and lipid peroxidation. Notably, such cellular changes seem to be universal across NBIA with different genetic origin. Currently, most forms of NBIA are treated with iron chelators. However, the nature of cellular pathology suggest that NRF2 based therapeutics can offer better treatment options for NBIA. Rationale for NRF2 based therapeutics for NBIA will be discussed.

P16: Chronic Rhinosinusitis & NRF2: Inflammation, Oxidative Stress, and Biomarker Horizons
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Chronic rhinosinusitis (CRS) is a prevalent inflammatory disease of the upper airways, affecting ~12% of the population and presenting in distinct phenotypes: CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP). The latter includes non-steroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease (N-ERD), a severe subtype associated with asthma and NSAID hypersensitivity. CRSwNP is predominantly driven by type 2 inflammation, while CRSsNP involves type 1 and type 3 immune mechanisms. Accumulating evidence implicates oxidative stress as a significant contributor to CRS pathogenesis; however, its interplay with inflammatory pathways remains poorly defined. Nuclear factor erythroid 2–related factor 2 (NRF2) orchestrates cellular antioxidant defences and may influence CRS by mitigating oxidative injury, modulating type 2 inflammation, and preserving epithelial barrier integrity. In murine models, Nrf2 deficiency exacerbates sinonasal inflammation and increases disease susceptibility, supporting its potential modulatory function In our research, we investigate the multifaceted role of NRF2 signalling in CRS using biobank-derived patient samples, high-throughput molecular profiling, and integration of published data. We further aim to characterise both established and novel NRF2-associated targets relevant to CRS pathophysiology. Identifying robust biomarkers of NRF2 activity in non-invasive biofluids such as nasal secretions, could inform precision approaches for difficult-to-treat CRS, including glucocorticoid-resistant disease and biologic non-responders. Our findings aim to pave the way for therapeutic strategies leveraging NRF2 activation in the sinonasal mucosa, potentially redefining management paradigms for chronic airway inflammation.

P17: Endogenous Nrf2-mediated oxidative stress response is not activated in mouse models of Crohn's Disease.

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Crohn's Disease (CD), one type of Inflammatory Bowel Disease (IBD), is a chronic recurrent intestinal inflammation associated with the loss of immune tolerance to intestinal microbiota that often affects ileum. It is a multifactorial disease with unknown cause and no long-term cure. Oxidative stress strongly contributes to CD pathology. Nrf2-mediated oxidative stress response has a potential to reduce the severity of CD due to its ability to restore redox balance and inhibit inflammation. Nrf2 is normally induced by Reactive Oxygen Species (ROS) and electrophiles, many of which are known to be produced in IBD. It is however not clear whether endogenous Nrf2-mediated oxidative stress response is in fact activated in CD, and whether such induction of Nrf2 can provide protection against CD and its complications. To find out if ileal inflammation in CD can by itself induce Nrf2 pathway, we have analysed the expression of several Nrf2 target genes in two mouse models of ileitis using qPCR screen. The short-term acute ileitis was induced in wild-type mice by intraperitoneal injection of anti-CD3 antibodies that causes T-cell-mediated self-resolving intestinal inflammation. The chronic CD-like ileitis was spontaneously developed in mice overproducing TNF due to a heterozygous deletion of regulatory ARE element in TNF gene (TNFdeltaARE mice). Surprisingly, neither of these models have shown a clear

activation of Nrf2 pathway. While some of Nrf2 targets were induced during ileal inflammation, others were unaffected or even suppressed. These results suggest that Nrf2 pathway might be actively inhibited during ileal inflammation.

P18: NRF2-independent role of KEAP1 in actin cytoskeleton regulation

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While the role of NRF2-dependent antioxidant responses is established in the protection against chronic diseases, emerging research reveals novel roles and functions for KEAP1, the primary regulator of NRF2. Our research focuses on novel, NRF2-independent functions of KEAP1 in regulating the cytoskeleton, which carries significant implications for the pathogenesis and management of chronic disease and cancer. KEAP1 interacts with the actin cytoskeleton through its double glycine repeat domain, forming a scaffold that sequesters NRF2. Recent findings suggest that KEAP1 may be involved in cytoskeleton regulation independently of its role in NRF2 degradation. To investigate this, we performed siRNA-mediated knockdown in cervical carcinoma HeLa and lung carcinoma A549 cell lines, followed by immunofluorescence or biochemical isolation of integrin adhesion complexes. KEAP1 depletion resulted in near-complete disassembly of actin stress fibers and significant reduction in both the number and size of focal adhesions, alongside decreased levels of key focal adhesion proteins. Functionally, cell migration was differentially affected: it increased in HeLa cells but decreased in A549 cells. Importantly, these effects were observed in both HeLa cells with intact KEAP1-NRF2 regulation and A549 cells harboring constitutively active NRF2, indicating NRF2-independent mechanisms. Our results expand the current understanding of KEAP1, revealing novel roles beyond its canonical regulation of NRF2. These findings have implications for chronic diseases, such as those involving inflammation, and cancers where cytoskeletal dysregulation contributes to the metastatic phenotype. Investigating NRF2-independent KEAP1 functions provide novel therapeutic targets alongside traditional strategies aimed at NRF2 activation.

P19: Advancements of young researchers within BenBedPhar COST Action on NRF2-targeted knowledge: Refining Redox Regulation for Therapeutic Applications

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The COST Action CA20121 – Bench to Bedside Transition for Pharmacological Regulation of NRF2 in Non-Communicable Diseases (BenBedPhar) has significantly contributed to advancing the careers and scientific development of young researchers. Through structured programs such as training schools, short-term scientific missions, workshops, and networking events, BenBedPhar has enabled young researchers to gain exposure to cutting-edge technologies, engage in cross-sectoral collaborations, and receive mentorship from established experts in redox biology and drug development. These opportunities have

further contributed to skill acquisition in experimental design, data analysis, and scientific dissemination. In example, an important practical finding for young researchers was the notation that NRF2 migrates anomalously in SDS-PAGE, appearing at ~95–110 kDa rather than its predicted 56–68 kDa. Misidentifying the lower band remains a common source of error, highlighting the need for precise detection to ensure reliable NRF2 research outcomes. Advancement in the mechanistic understanding of the NRF2 pathway is also a valuable step forward in therapeutic targeting of NRF2. Earlier models suggested that NRF2 inducers merely caused conformational changes in KEAP1, leading to a closed complex in which NRF2 remained trapped and only newly synthesized NRF2 could undergo nuclear translocation. However, this view does not fully account for the complexity of NRF2 regulation. More recent insights reveal that NRF2 stability and activity are governed by multiple ubiquitin ligases, which act in a stress- and cell-type–dependent manner. Furthermore, endogenous soft electrophiles activate NRF2 by chemically modifying KEAP1 redox switches, underscoring a dynamic and multilayered regulatory system. From a practical perspective for ECI this shift in understanding emphasizes the need to design experimental approaches and therapeutic strategies that account for the diverse mechanisms controlling NRF2 stability and activation.

P20: NRF2 and arachidonic acid mediators in neurodegenerative diseases

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Background: Elevated levels of arachidonic acid (AA) derivatives have been reported in several neurodegenerative diseases. In contrast, nuclear factor (erythroid-derived 2)-like2 (Nrf2) exerts neuroprotective and anti-inflammatory effects. Given these opposing roles, we aim to investigate the correlation between AA derivatives and NRF2 in Parkinson's disease, Alzheimer's disease, and multiple sclerosis. Methods: Pubmed and Scopus databases were used to identify all the studies investigating the correlation between AA mediators and NRF2 in neurodegenerative diseases, particularly in Parkinson, Alzheimer, multiple sclerosis. Results: AA and NRF2 work together to control inflammation and oxidative stress. Some AA mediators, like 15d-PGJ_2 and other LOX-derived molecules, can activate NRF2, which turns on protective antioxidant genes (example HO-1). Once active, NRF2 reduces inflammation by lowering COX-2 and iNOS levels and decreasing the production of prostaglandins (PGs), thromboxane (TX), and leukotrienes (LT). It also boosts antioxidant defenses, preventing lipid damage and cell death. In Alzheimer's, Parkinson's, and multiple sclerosis, activating NRF2 protects neurons, reduces glial inflammation, and limits oxidative stress. Conclusion: NRF2 and arachidonic acid derivatives are tightly interconnected in controlling oxidative stress and inflammatory responses with AA metabolites activating NRF2 and NRF2 limiting AA-driven inflammation and oxidative damage. In conclusion, modulating AA pathways, such as by inhibiting COX-2 or 5-LOX, may affect NRF2 activity and provide additional ways to reduce neuroinflammation and protect neurons in neurodegenerative diseases.

P21: Cholesterol-Induced Lipid Accumulation in Hepatocytes: Insights from NRF2 Modulation

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Hepatic steatosis, characterized by abnormal lipid accumulation in the liver, is the most common type of metabolic-associated fatty liver disease (MAFLD), with its prevalence increasing globally. In addition to fatty acids, cholesterol-related hepatic lipid droplet formation, and the induction of oxidative stress, inflammation, and cell death, plays a key role in the development of metabolic dysfunction-associated steatohepatitis (MASH). While many studies have extensively revealed the protective role of nuclear factor erythroid 2-related factor 2 (NRF2) in MASH progression, its specific role in cholesterol-driven hepatic lipid accumulation remains poorly defined. In the present study, we established an in vitro model of lipid accumulation using cholesterol-rich liposomes. NRF2 activity was modulated in two ways: by silencing Keap1 with siRNA, and by direct NRF2 knockdown with siRNA. Changes in lipid droplet number and size were determined by confocal microscopy, and oxidative stress was measured by flow cytometry. Additionally, protein expression of apoptosis-related parameters, including caspase 3 and caspase 9 was determined by western blotting. Collectively, these results highlight NRF2 as a critical regulator of cholesterol-induced hepatic steatosis and suggest that targeting NRF2 signaling may provide new therapeutic strategies to prevent the onset and progression of MASH in individuals with high cholesterol.

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P22: Nrf2 knockout mice as a tool to study CNS autoimmunity

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Studying roles of Nrf2 in autoimmunity, as well as opportunities to use Nrf2 modification for the treatment of autoimmune disorders is highly important. Nrf2 knockout (ko) mice have been extensively used in such studies. Here, relevance of Nrf2 ko mice in the research of CNS autoimmunity, *i.e.* in experimental autoimmune encephalomyelitis (EAE) will be discussed. Specifically, influence of Nrf2 ko on EAE clinical course, as well as on functional properties of immune cells therein will be addressed. The results of other groups, as well as our own, suggest that Nrf2 ko mice are a valuable tool for studying CNS autoimmunity, yet with important limitations.

P23: Kyn-CKA engages KEAP1 Cys151 to reprogramme macrophage inflammation via NRF2

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Chronic inflammatory diseases are characterised by macrophage-driven oxidative stress and persistent cytokine signalling. Precise activation of NRF2 in immune cells has remained challenging. In this study, direct engagement of KEAP1 by L-kynurenine-derived Kyn-CKA at Cys151 was established, and increased KEAP1 thermal stability was demonstrated by thermal shift assays. In KEAP1 cysteine-mutant MEFs, abolition of NRF2 accumulation and NQO1 induction was observed upon loss of Cys151, whereas responsiveness was retained in other mutants. In primary BMDMs, LPS-evoked pro-inflammatory transcription was attenuated and NRF2 targets were restored after Kyn-CKA pre-treatment; these effects were absent in Nrf2-knockout macrophages and enhanced in Keap1-knockdown models. By linking tryptophan catabolism to KEAP1 sensing, a metabolite-to-sensor mechanism was defined that harnesses NRF2 in macrophages for chronic conditions with oxidative stress and macrophage dysregulation, in which IDO1 activity is frequently elevated, including sickle cell disease and inflammatory bowel disease.

P24: Differential Sensitivity of Breast Cancer Cell Lines to H_2O_2-Induced Oxidative Stress: Roles of AMPK and the NRF2 Pathway

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Breast cancer is the most prevalent malignancy among women and is characterized by considerable heterogeneity. It is commonly classified by hormone receptors (HR) and HER2 status into HR-positive, HER2-positive, and triple-negative breast cancer (TNBC). Conventional treatments such as radio- and chemotherapy remain central, particularly for TNBC, which lacks effective targeted options. These therapies also rely on the induction of oxidative stress (OS) by exploiting the increased levels of reactive oxygen species (ROS) found in cancer cells. The NRF2 pathway is a major regulator of antioxidative defense, while AMPK acts as a sensor of metabolic stress; both, together with ROS, play context-dependent roles in cancer progression and therapeutic resistance. We examined whether the duration of H_2O_2-induced OS (1 vs. 7 days) influences adaptive responses in three breast cancer cell lines representing distinct subtypes. Viability, proliferation, intracellular ROS, antioxidative capacity, and AMPK involvement were assessed. The cell lines displayed distinct sensitivities to OS. TNBC SUM159 cells were the most tolerant, exhibiting adaptation upon prolonged exposure, which was associated with aberrant NRF2 signaling and AMPK activation, in connection with NQO1 and HO-1.

P25: Dimethyl fumarate decreases plasma triglyceride level in female hypertriglyceridemic rats exposed to chronic psychosocial stress

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Stress is a significant factor involved not only in the development, but also in the progression of non-communicative diseases, especially in individuals with a genetic predisposition. This study investigated the effects of chronic social stress on systolic blood pressure (BP), heart rate (HR), plasma triglyceride (TG) levels and expression of Nfe2l2 and selected NRF2-target genes in the heart and liver of female hypertriglyceridemic (HTG) rats. Additionally, the effects of dimethyl fumarate (DMF), an NRF2 activator, were examined. Four groups of adult female HTG rats were investigated: 1) control rats (treated with vehicle, 0.25% DMSO, p.o.), 2) stress-exposed rats (4-week crowding, 5 rats/cage, 182 cm^2/rat), 3) DMF-treated rats (20 mg/kg/day in the vehicle for 4 weeks, p.o.), and 4) rats exposed to 4 weeks of stress simultaneously with DMF treatment. In contrast to male HTG rats, stress and DMF had no effect on SBP and HR measured by the tail-cuff method. The TG levels in control female rats were approximately 5.9 mmol/L. DMF significantly reduced TG levels and atherogenic index of plasma in stress-exposed rats compared to the stress-only group. The effects on NRF2 expression in the tissues will also be presented. In conclusion, the results indicate that DMF treatment reduced stress-induced increases in TG levels in female HTG rats, similarly as was found in male rats, suggesting therapeutic potential of DMF in the treatment of hypertriglyceridemia. Supported by grant 09I03-03-V04-00477.

P26: Mechanistic Insights into Garcinoic Acid's Regulation of Cholesterol-Induced Steatosis: Role of NRF2/HO-1 Axis

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The prevalence of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) is steadily increasing worldwide in parallel with the rise of hepatic lipid accumulation. Garcinoic acid, a natural derivative of delta-tocotrienol (δ-T3), is a developing area of interest in regulating various process, including lipid homeostasis and inflammation. However, its involvement on cholesterol-induced hepatic lipid accumulation and role of NRF2/HO-1 pathway remains poorly defined. In the present study, we established an in vitro lipid accumulation model by incubating AML12 cell line with cholesterol-rich liposomes and administrated garcinoic acid. NRF2 activity was silenced by siRNA transfection and HO-1 inhibition was achieved by Zinc Protoporphyrin-IX (Znpp-IX) administration. Changes in lipid droplet number and size were determined by confocal microscopy, while protein and mRNA expressions of various lipid homeostasis and inflammation parameters, including SREBP1c, FASN, SCD, ACC, ACLY, GPAT and ACS; TNF-α, TGF-β, IL-6, IL-1β and IL-10; were determined by western blotting and qRT-PCR. Our findings demonstrate that garcinoic acid effectively reduces cholesterol-induced lipid droplet maturation, along with the expression of genes related to lipid metabolism and inflammation. Both NRF2 silencing and HO-1 inhibition also modulated these processes. Taken together, these results reveal a novel role of garcinoic acid in mediating cholesterol-induced lipid accumulation and encourage further studies exploring its therapeutic potential against NAFLD.

P27: DPP3 Knockdown Impairs Cell Migration and Cell Cycle Progression in HeLa Cells

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Dipeptidyl peptidase 3 (DPP3) is a zinc metallopeptidase that cleaves dipeptides from the N-termini of 3–10 amino acids long peptides. It has been proposed to participate in the final stages of intracellular protein turnover and may also play a role in the regulation of blood pressure and pain. Additionally, DPP3 upregulates NRF2 activity through competitive binding to KEAP1. DPP3 is overexpressed in various cancer tissues compared with normal counterparts, suggesting its involvement in tumour development; however, the underlying mechanisms remain unclear. More recent studies have also identified DPP3 as a prognostic marker and potential therapeutic target in septic and cardiogenic shock, where the increased levels of circulating DPP3 were correlated with higher mortality risk. We knocked down DPP3 expression in HeLa cells using siRNA and found that DPP3 depletion inhibited cell migration in a wound healing assay. Cell cycle analysis by propidium iodide (PI) staining and flow cytometry revealed that a lower proportion of DPP3-knockdown cells progressed to S phase compared with siRNA control cells. Consistent with these findings, DPP3-knockdown cells showed increased protein expression of p21 (CDKN1A) and decreased levels of cyclin A and cyclin E. In contrast, expression of the NRF2 target gene NQO1 was not affected at either the protein or mRNA level, suggesting that basal NRF2 activity is not impaired by DPP3 knockdown, or that compensatory mechanisms increase NRF2 expression. Considering the frequent overexpression of DPP3 in cancer, these results may have implications for the development of DPP3-targeted cancer therapies.

P28: PIN1-NRF2 Axis: Characterization of interaction, Pharmacological Inhibitors, Preliminary Implications from Endothelial Aging Models

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PIN1, a peptidyl-prolyl isomerase, has been implicated in diverse cellular processes, including proliferation and stress adaptation. In the context of redox regulation, PIN1 interacts with the transcription factor NRF2, yet the molecular determinants and biological implications of this axis remain incompletely defined.

Our computational analyses identified key residues in the PIN1 WW domain (Ser16, Arg17, Ser18, Tyr23, Ser32, Gln33, and Trp34) as critical for PIN1-NRF2 protein-protein interactions (PPIs). Fluorescence polarization (FP) assays confirmed that Pintide, a peptide targeting the WW domain, significantly disrupted the binding of NRF2-mimicking peptides. Furthermore, pharmacological profiling revealed that juglone, EGCG, and KPT-6566 attenuated PIN1-NRF2 interactions, with KPT-6566 exhibiting the most potent effect. Mass spectrometry suggested that KPT-6566 covalently modified PIN1 through conjugate addition rather than disulfide exchange. Complementing these mechanistic findings, preliminary cellular data from human aortic endothelial cells (HAEC) indicated that PIN1 expression was substantially higher both in the cytoplasm and in the nucleus of aged donors (>70 years) compared with young donors (<25 years). In NRF2-silenced HAEC, mimicking premature senescence, PIN1 localization was further increased both in the nucleus and cytoplasm relative to controls, resembling the patterns observed in physiological aging. Together, these data suggest a reciprocal regulatory relationship where loss of NRF2 is accompanied by enhanced PIN1 expression and nuclear localization. Our integrated molecular and cellular evidence highlights the PIN1-NRF2 axis as a potential target for pharmacological modulation in age-associated vascular dysfunction. Acknowledgment: T.B. Tumer acknowledges support from the Ulam Fellowship of the Polish National Agency for Academic Exchange (NAWA; BPN/ULM/2023/1/00133/U/00001).

P29: Cardioprotective role of sirtuin1-deacetylase and Nrf2 axis in high-sucrose-diet-induced metabolic syndrome mouse

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Background and aim: Nuclear factor-erythroid 2-related factor 2 (Nrf2) regulates oxidative stress by inducing antioxidant elements upon nuclear translocation. Sirtuin 1 (Sirt1) may activate Nrf2 via deacetylation. Insulin resistance in metabolic syndrome alters protein activity through post-translational modifications, such as lysine acetylation. This study investigates the effects of insulin resistance on Nrf2 and Sirt1. Materials & Methods: In vivo, male Balb/c mice were fed a 32% sucrose diet for 15 weeks to induce insulin resistance. In vitro, H9c2 cells and isolated cardiomyocytes were treated with 50 µM palmitic acid for 24 h. Control cardiomyocytes were incubated with Sirt1 inhibitor EX527 (10 µM), while insulin-resistant cardiomyocytes received Sirt1 activator SRT1720 (2 µM). Nrf2 and Sirt1 levels and K-acetylation were analyzed via Western blotting. Mitochondrial membrane potential (MMP), reactive oxygen species (ROS), and intracellular zinc/calcium ($[Zn^{2+}]i$, $[Ca^{2+}]i$) were measured using fluorescence techniques. Results: Insulin-resistant H9c2 cells and mouse cardiomyocytes exhibited elevated K-acetylation. Nrf2 and Sirt1 levels decreased in insulin-

resistant groups, alongside MMP depolarization, increased ROS, and elevated [Zn^{2+}]i and [Ca^{2+}]i. SRT1720 incubation mitigated these effects.
Conclusion: Sirt1 inhibition downregulates Nrf2, exacerbating ROS production and MMP disruption. Sirt1 activation might counteract insulin resistance-induced oxidative stress.

P30. In Silico Design of Investigational Drugs Targeting ERα and BRCA1 Proteins for Breast Cancer Therapy

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Breast cancer continues to be a major cause of cancer-related death globally and is one of the most common cancers in women. Estrogen receptor alpha (ERα), a key modulator of hormone-dependent tumor growth, and BRCA1, a tumor suppressor necessary for DNA repair and genomic integrity, are strongly linked to its pathogenesis. Changes in these proteins are intriguing targets for precision drug development because they play a role in tumor initiation, progression, and resistance to therapy. Searching small compounds that efficiently interact with ERα and BRCA1 may lead to better therapeutic results when treating breast cancer. In this study, the crystal structures of ERα and BRCA1 were retrieved from the Protein Data Bank (PDB), and the potential ligands were acquired from the ZINC20 database. AutoDock Vina was used to assess binding affinities and interaction types for molecular docking. Ligands with docking scores greater than -9.0 kcal/mol were chosen as candidates for additional analysis. Molecular dynamics (MD) simulation input files were created for these high-scoring complexes using the CHARMM-GUI interface, applying the proper force field, ionization, and solvation parameters. The constructed complexes were further put through 200 ns MD simulations using NAMD to examine structural stability and conformational dynamics to simulate the physiological conditions. The simulations were followed by post-MD analyses, which included solvent-accessible surface area (SASA) for solvent exposure, radius of gyration (Rg) for molecular compactness, root mean square deviation (RMSD) for overall structural stability, and root mean square fluctuation (RMSF) for local residue flexibility. ADMET estimations were also performed to evaluate pharmacokinetic and toxicological properties. In order to visualize molecular interactions, identify essential residues involved in binding, and compare the interaction patterns between ERα and BRCA1, BIOVIA Discovery Studio was utilized. The outcomes showed that the chosen ligands had excellent ADMET properties, little structural variations, and stable binding modes throughout the 200 ns simulations. These results provide an integrated, economical computational approach for experimental drug discovery and demonstrate the potent compounds as lead candidates for multi-target breast cancer therapy.

P31. NRF2-activating agents exert antioxidant effects by enhancing endogenous H_2S formation.

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Background: Nuclear factor erythroid 2–related factor 2 (NRF2) is a master regulator of cellular antioxidant defense. Hydrogen sulfide (H_2S) is an endogenously produced gaseous neurotransmitter, activates NRF2 by persulfidating critical cysteine residues of Keap1, thereby preventing NRF2 degradation and promoting its nuclear translocation to induce antioxidant gene expression [1]. H_2S donors exhibit neuro/cardioprotection in oxidative stress-related disorders in preclinical studies; but, their clinical use is limited by unpleasant odor and short half-lives [2,3]. However, endogenous H_2S stimulators with antioxidant properties have advantages as a promising therapeutic approach over H2S donors regarding safety and patient compliance. Empagliflozin (EMPA), a sodium-glucose cotransporter-2 (SGLT2) inhibitor, widely used in diabetes mellitus (DM) and sildenafil, a phosphodiesterase-5 inhibitor, both have antioxidant and neuroprotective effects and have been reported to activate the NRF2 signaling [4,5]. This study aimed to investigate whether endogenous H_2S production contributes to the neuroprotective and antioxidant effects of EMPA and sildenafil. Methods: Ex vivo experiments in mouse brain and heart tissues performed to assess the effects of EMPA on H_2S levels and reactive oxygen species (ROS) formation under basal and Pyrogallol-induced oxidative stress conditions. Rats were divided into four groups: nondiabetic, EMPA-treated nondiabetic, streptozotocin (STZ)-induced diabetic, and EMPA-treated STZ-diabetic. Brain H_2S and ROS levels were measured by methylene blue and chemiluminescence assays, respectively. The role of H_2S was further confirmed using the synthesis inhibitor aminooxyacetic acid (AOAA).

Results: Ex vivo EMPA and Sildenafil treatment significantly increased endogenous H2S formation in both healthy and pyrogallol-induced oxidative stress and reduced ROS formation in mouse brain and heart, respectively; these effects were significantly reversed by the H2S synthesis inhibitor, aminooxyacetic acid (AOAA)($p<0.001$, n=6). Oral EMPA administration significantly elevated brain H_2S levels in both nondiabetic and diabetic rats ($p<0.001$, $p<0.05$, n=6, respectively) and reduced ROS formation ($p<0.01$, n=6). These effects were inhibited by AOAA. Conclusions: Our findings suggest that NRF2-activating agents exert neuroprotection and cardioprotection against oxidative stress through the H_2S pathway. The NRF2–H_2S signaling axis highlights a novel mechanism with therapeutic potential in oxidative stress–related disorders. Acknowledgement: We thank the Turkish Scientific Research Council (TUBITAK) for the support (grant numbers: 119S769, 1919B012301772 and 1919B012320814).

References:

1. Yang, G.; Zhao, K.; Ju, Y.; Mani, S.; Cao, Q.; Puukila, S.; Khaper, N.; Wu, L.; Wang, R. Hydrogen sulfide protects against cellular senescence via S-sulfhydration of Keap1 and activation of Nrf2. Antioxid Redox Signal 2013, 18, 1906-1919, doi:10.1089/ars.2012.4645.
2. Tripathi, S.J.; Chakraborty, S.; Miller, E.; Pieper, A.A.; Paul, B.D. Hydrogen sulfide signalling in neurodegenerative diseases. Br J Pharmacol 2023, doi:10.1111/bph.16170.
3. Yang, F.; Zhang, L.; Gao, Z.; Sun, X.; Yu, M.; Dong, S.; Wu, J.; Zhao, Y.; Xu, C.; Zhang, W.; et al. Exogenous H2S Protects Against Diabetic Cardiomyopathy by Activating Autophagy via

the AMPK/mTOR Pathway. Cell Physiol Biochem 2017, 43, 1168-1187, doi:10.1159/000481758.
4. Shi, P.; Zhan, Z.; Ye, X.; Lu, Y.; Song, K.; Sheng, F.; Shen, H.; Yin, P. The antioxidative effects of empagliflozin on high glucose-induced epithelial-mesenchymal transition in peritoneal mesothelial cells via the Nrf2/HO-1 signaling. Ren Fail 2022, 44, 1528-1542, doi:10.1080/0886022X.2022.2118066.
5. Zahran, M.H.; Hussein, A.M.; Barakat, N.; Awadalla, A.; Khater, S.; Harraz, A.; Shokeir, A.A. Sildenafil activates antioxidant and antiapoptotic genes and inhibits proinflammatory cytokine genes in a rat model of renal ischemia/reperfusion injury. Int Urol Nephrol 2015, 47, 1907-1915, doi:10.1007/s11255-015-1099-5.

P32. Phytochemical characterization of *Rhodiola rosea* L and its therapeutic effect on Alzheimer's disease

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Rhodiola rosea L. is known for its adaptive, anti-depressive, immune stimulating and apoptosis-inducing activities in glioma and human neuroblastoma cells[1]. The most valuable molecules in this plant are found in its rhizomes[2]. Limited number of studies reveal that *R. rosea* might have beneficial effect in degenerative neurological disease, including Alzheimer's disease (AD)[3]. However, its effect on NRF2 in AD has not been investigated. The aim of the study was to perform phytochemical analysis of *R. rosea* rhizomes, wild-grown in Bulgaria and study its neuroprotective effect in AD. The obtained one- and two-dimensional nuclear magnetic resonance (NMR) spectra revealed the presence of the major phenylethanoids (p-tyrosol and salidroside) and phenylpropanoids (rosin, rosavin and rosarin). Functional analysis in the *Caenorhabditis elegans* (*C. elegans*) AD transgenic strain (CL2006) showed that *R. rosea* upregulated *sod-1/SOD-1* and *skn-1/NRF2*, two central regulators of oxidative stress defense. These effects appear to be associated with modulation of the insulin/IGF-1 pathway through *daf-2/IGF1R*, further supporting enhanced stress resistance and longevity signaling. Moreover, behavioral assays revealed that *R. rosea* extracts significantly delayed Aβ-induced paralysis in CL2006 worms compared to untreated controls, highlighting a functional neuroprotective effect. The treatment with *R. rosea* extract reduced the accumulation of reactive oxygen species (ROS) over time, reaching levels similar to the antioxidant reference vitamin C. These findings suggest that *R. rosea* activates protective molecular pathways related to oxidative stress and insulin-like signaling, and functionally mitigates Aβ toxicity, thereby strengthening its potential as a candidate for neurodegenerative disease intervention.

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1. Marchev, A. S., Dimitrova, P., Koycheva, I. K., Georgiev, M. I. (2017). Altered expression of trail on mouse T cells via Erk phosphorylation by *Rhodiola rosea* L. and its marker compounds. Food and Chemical Toxicology, 108, 419-428

2. Marchev, A. S., Koycheva, I. K., Aneva, I. Y., Georgiev, M. I. (2020). Authenticity and quality evaluation of different *Rhodiola* species and commercial products based on NMR-spectroscopy and HPLC. Phytochemical Analysis, 31(6), 756–769.

3. Zhang, B., Zhang, X., Treebuphachatsakul, W., Pantan, R., Kampan, N., Phatsara, M., Shi, C., Narakornsak, S. (2025). Salidroside alleviates Aβ1–42-induced ferroptosis in human neuroblastoma cells by AKT/gsk-3β activation via inhibiting VDAC1. Phytomedicine Plus, 5(3), 100802.

P33. Nox4 is involved in acute kidney injury associated to intravascular hemolysis

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Massive intravascular hemolysis occurs not unfrequently in many clinical conditions. Breakdown of erythrocytes promotes the accumulation of heme-derivates in the kidney, increasing oxidative stress and cell death, thus promoting acute kidney injury (AKI). NADPH oxidase 4 (Nox4) is a major source of reactive oxygen species (ROS) in the kidney, however it is unknown the role of Nox4 in hemolysis and whether inhibition of this enzyme may protect from heme-mediated injury. To answer these questions, we elicited intravascular hemolysis in wild type and Nox4 knockout mice. We also evaluated whether nephrotoxic effects of heme may be reduced by using Nox4 siRNA and pharmacologic inhibition with GKT137831, a Nox4 inhibitor, both in vivo and in cultured renal cells. Our results showed that induction of massive hemolysis elicited AKI characterized by loss of renal function, morphological alterations of the tubular epithelium and podocytes, oxidative stress (including Nrf2 activation and increased HO-1 expression), inflammation, mitochondrial dysfunction, blockade of autophagy and cell death. These pathological effects were significantly prevented in Nox4-deficient mice and in animals treated with GKT137831. In vitro studies showed that Nox4 disruption by specific siRNAs or Nox4 inhibitors declined heme-mediated ROS production and cell death. Our data identify Nox4 as a key enzyme involved in intravascular hemolysis-induced AKI. Thus, Nox4 inhibition may be a potential therapeutic approach to prevent renal damage in patients with severe hemolytic crisis.