

synthesis of purine bases. This interaction is shared across MERS, SARS-CoV-1, and SARS-CoV-2, making it a potential target for pan-coronavirus antivirals. We recombinantly produced and purified both proteins in *E. coli* and analysed the enzymatic activity of hIMPDPH2 in the presence of Nsp14. The results demonstrated a linear increase in hIMPDPH2 activity with rising concentrations of Nsp14. This aligns with previous findings of enhanced production of nucleotides and purine-base precursors in *in vitro* cellular experiments. The binding affinity of Nsp14 with the untagged hIMPDPH2, as measured using surface plasmon resonance, was in the low micromolar range. However, binding was lost when an N-terminal His-tagged construct of hIMPDPH2 was used. Furthermore, contrary to the untagged hIMPDPH2, the enzymatic activity of His-tagged hIMPDPH2 was not enhanced by Nsp14, suggesting that an intact N-terminus of hIMPDPH2 is necessary for Nsp14 binding and hIMPDPH2 enzymatic activity enhancement. Consistent with these observations, structure prediction of the Nsp14-hIMPDPH2 complex using AlphaFold Multimer revealed a model where the N-terminus of hIMPDPH2 binds to a central groove of Nsp14, thus rationalizing the loss of binding and the enzymatic activity enhancement when His-hIMPDPH2 is utilized. Our functional and *in silico* structural prediction analysis provide a model of the Nsp14-hIMPDPH2 complex, to be validated through experimental structural analysis. Additionally, this model reveals a binding interface to be targeted for the design of pan-coronavirus antivirals.

P-36-064

Targeting lipid peroxidation in cystic fibrosis as a new approach to assist current therapy

I. Artusi^{1,II}, M. Rubin^I, A. Pianazzola^I, V. Bosello-Travain^I, G. Cozza^I

^IDepartment of Molecular Medicine, University of Padova, Padova, Italy, ^{II}Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy

Cystic fibrosis (CF) is an inherited disorder caused by mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride/bicarbonate channel located in different tissues and notably in the lungs. Here, CF is responsible for airways obstruction by viscous mucus and chronic inflammation. Approved therapies are addressed to a limited numbers of mutations consequently the search for new therapeutic proposals is still urgent. The most common variant, F508del, is responsible for misfolding, ER accumulation and premature degradation of the channel preventing its trafficking to the plasma membrane. This results in ER stress, persistent inflammation and oxidative imbalance related to an overproduction of ROS. Specifically, a marked depletion of glutathione (GSH), as well as of its synthesis regulator NF-E2-related factor 2 (Nrf2), is observed in CF cell lines which fosters the oxidative damage of polyunsaturated fatty acids (PUFA) via lipid peroxidation (LPO). LPO may evolve in ferroptosis, an iron-mediated form of cell death. We detected high ROS and LPO levels in cells expressing F508del-CFTR (CFBE41o- parental) compared to WT (16hBE). Based on that, we aim to improve mutated CFTR proteostasis and function by restoring the redox homeostasis. Indeed, we analysed molecules with specific radical-trapping activity demonstrating a decrease of LPO, as observed through C11-Bodipy technique, and a protection of CF cell lines from erastin-induced ferroptotic cell death. Furthermore, these compounds, in specific combinations with current therapies, increase

channel trafficking at the PM and reinstate CFTR-mediated ion transport in CFBE epithelia providing an enhanced effect in Ussing chamber experiments. The obtained data suggest a novel approach that could implement existing clinical therapies. Being not directed against the mutated CFTR, this proposal holds the potential to be translated to orphan mutations.

P-36-065

Effect of D-ribose on glycation of human high-density lipoprotein

C. Morresi^{*I}, T. Bacchetti^{*I}, G. Ferretti^{*II}

^IDepartment of Life and Environmental Sciences (DiSVA), Polytechnic University of Marche, Ancona, Italy, ^{II}Department of Clinical Sciences, Università Politecnica delle Marche, Ancona, Italy

Advanced glycation end products (AGEs) have significant role in the pathophysiology of diabetes. Glucose, fructose and methylglyoxal trigger glycation of plasma lipoproteins as we previously demonstrated. D-ribose is a naturally occurring pentose monosaccharide present in all living cells and their microenvironments and is a key component of numerous biomolecules involved in many important metabolic pathways. It also participates in the glycation of proteins, producing AGEs that lead to cell dysfunction and death. Recent studies show that incubation with D-ribose causes protein aggregation and alterations of their functional activities. No report has been previously published to investigate D-ribose induced glycation (ribosylation) of high-density lipoprotein (HDL). The aim of the study was to investigate the effect of *in vitro* ribosylation on human HDL composition and functions; in particular, the activity of the HDL surface-associated enzyme paraoxonase-1 (PON1) and HDL antioxidant properties, were investigated using the dihydrorhodamine 123 (DHR) assay. The significant increase in fluorescent AGEs after incubation with D-ribose, confirms that human HDL is susceptible to ribosylation. The decrease of free amino groups and of intrinsic fluorescence of tryptophan demonstrates HDL apoprotein modifications in ribosylated lipoproteins. The compositional changes are associated with a significant decrease of PON1 activity and impaired HDL antioxidant properties. Our results provide evidence that ribose triggered glycation of HDL interferes in their normal physiological functions and might contribute to diabetic complications such as atherosclerosis and other cardiovascular diseases. *The authors marked with an asterisk equally contributed to the work.

P-36-066

Insulin signaling is critical for sinoatrial node maintenance and function

J. Kim, S. Ock, W. Lee

Chung-Ang University, Seoul, South Korea

Insulin and insulin-like growth factor 1 (IGF-1) signaling regulate cellular growth and glucose metabolism in the myocardium. However, their physiological role in the cells of the cardiac conduction system has never been explored. Therefore, we sought to determine the spatiotemporal function of insulin/IGF-1 receptors in the sinoatrial node (SAN). We generated cardiac conduction cell-specific inducible IGF-1 receptor (IGF-1R) knockout (KO) (CSIGF1RKO), insulin receptor (IR) KO (CSIRKO), and IR/IGF-1R double-KO (CSDIRKO) mice and evaluated their

phenotypes. Telemetric electrocardiography revealed regular sinus rhythm in CSIGFIRKO mice, indicating that IGF-1R is dispensable for normal pacemaking. In contrast, CSIRKO and CSDIRKO mice exhibited profound sinus bradycardia. CSDIRKO mice showed typical sinus node dysfunction characterized by junctional rhythm and sinus pauses on electrocardiography. Interestingly, the lack of an insulin receptor in the SAN cells of CSIRKO and CSDIRKO mice caused sinus nodal fibrosis. Mechanistically, hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) protein expression significantly decreased in the CSIRKO and CSDIRKO mice relative to the controls. A patch-clamp study of the SAN cells of CSIRKO mice revealed a significant decrease in the funny current, which is responsible for spontaneous diastolic depolarization in the SAN. This result suggested that insulin receptor loss reduces the heart rate via downregulation of the HCN4 channel. Additionally, HCN1 expression was decreased in CSDIRKO mice, explaining their sinus node dysfunction. Our results reveal a previously unrecognized role of insulin/IGF-1 signaling in sinus node structural maintenance and pacemaker function.

P-36-067

N-acetylaspartate promotes glycolytic-to-oxidative fiber-type switch in myotubes

S. Castelli^I, E. Desideri^{II}, L. Laureti^I, M.R. Ciriolo^{I,III}, F. Ciccarone^{I,III}

^IUniversity of Rome "Tor Vergata," via della ricerca scientifica 00133, Rome, Italy, ^{II}San Raffaele Open University, via di val Cannuta 247 00166, Rome, Italy, ^{III}IRCCS San Raffaele Roma, via di val Cannuta 247 00166, Rome, Italy

N-acetylaspartate (NAA) is an N-acetylated derivative of aspartate and stands as the second most abundant metabolite in CNS, reaching concentrations of approximately 10 mM. NAA is almost exclusively synthesized in neurons by the mitochondrial enzyme aspartate N-acetyltransferase 8-like (NAT8L) starting from aspartate and acetyl-CoA. It is widely used as a marker for neuronal integrity/function, and its brain concentration promptly decreases in neurodegenerative disorders while increasing in the circulatory blood system [Previously published in: Schuff N. et al. (2006) *Adv Exp Med Biol.* 576: 241–363]. Although NAT8L is primarily expressed in CNS, aspartoacylase (ASP), which catabolizes NAA into free acetate and aspartate, is more widely distributed in most peripheral tissues [Previously published in: Bogner-Strauss JG. (2017) *Front Endocrinol (Lausanne)* 8:240]. We focused our attention on the skeletal muscle, which is frequently altered in patients affected by neurodegenerative disorders, such as motor neuron diseases. In particular, amyotrophic lateral sclerosis (ALS) induces a change in muscle metabolic phenotype passing from glycolytic-to-oxidative fiber types [Previously published in: Scaramazza S. (2020) *iScience* 23 (5):101087]. Our results indicate that treatment of differentiated myotube with NAA induces this type of metabolic switch by enhancing lipid turnover. The importance of NAA catabolism was demonstrated by the use of CRISPR-Cas9 ASPA knockout muscle cells. Overall, we hypothesize that NAA accumulation in the blood of patients suffering neurodegenerative diseases may contribute to brain-body signalling and impact the metabolism and adaptive response of the skeletal muscle.

P-36-068

COVID-19 induces cell surface-associated fibrinolysis shutdown

T. Yatsenko^{I,II}, R. Rios^{III}, T. Nogueira^{III}, Y. Salama^{IV}, E. Adachi^V, S. Takahashi^{VI}, K. Hattori^{VII}, B. Heissig^{II}

^IPalladin Institute of Biochemistry of NAS of Ukraine, Kyiv, Ukraine, ^{II}Department of Bioresource Bank, Graduate School of Medicine, Juntendo University, Tokyo, Japan, ^{III}Institute of Computing, Federal University of Bahia, Salvador-Bahia, Brazil, ^{IV}An-Najah Center for Cancer and Stem Cell Research, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestinian Territories, ^VInstitute of Medical Science, the University of Tokyo, Tokyo, Japan, ^{VI}Division of Clinical Precision Research Platform, the Institute of Medical Science, the University of Tokyo, Tokyo, Japan, ^{VII}Center for Genome and Regenerative Medicine, Juntendo University, Tokyo, Japan

Hypercoagulation, thrombosis, and ARDS are typical complications in patients with COVID-19. The thrombotic tendency is often linked to increased plasminogen activator inhibitor-1 (PAI-1) levels and a concomitant suppression of fibrinolysis. In the present study, we examined changes in cell- and fibrin-associated fibrinolysis markers, their correlation with inflammatory factors and determined the predictive impact of urokinase and tissue-type plasminogen activators (uPA and tPA) and PAI-1 as a prognostic biomarker for COVID-19 severity. The cohort included 69 Japanese adults admitted to hospitals due to COVID-19 and 20 healthy controls. We found elevated free, non-complexed PAI-1 antigen, low circulating uPA, and uPA/PAI-1 but not tPA/PAI-1 complex levels associated with COVID-19 severity and ARDS development. This biomarker profile was typical for patients in the complicated phase. There was a lack of PAI-1 activity in circulation despite free, non-complexed PAI-1 protein. Furthermore, plasmin/ α 2antiplasmin complex – known to indicate active plasmin – correlated with soluble uPAR and soluble VCAM levels, markers indicating endothelial dysfunction. uPA/PAI-1 complex levels positively correlated with TNF α , a cytokine reported to trigger inflammatory cell death and tissue damage. Those levels also positively correlated with the pro-inflammatory factors interleukin1b (IL1b), IL6, and C-reactive protein, markers associated with the anti-viral inflammatory response. Reductions in uPA, a crucial protease for cell-associated plasmin production, and uPA/PAI-1 complex, alongside increased plasmin/ α 2antiplasmin complexes, indicate activation of fibrinolysis in circulation, but suppression of pericellular plasmin generation in individuals diagnosed with COVID-19. The finding that the disease severity correlates with cell-associated fibrinolysis shutdown argues for using uPA and uPA/PAI-1 as novel biomarkers to detect patients at risk of developing severe COVID-19, including ARDS.