(WT) or mice lacking functional Nrf2 (Nrf2KO) to further investigate the role of Nrf2 in the local and systemic two-way redox communication between breast cancer and adipose tissue (AT). To this end, we examined changes in GSH levels as well as the activity of the GSH-dependent enzymes in tumor tissue, CAAT, and distant AT in $Nrf2^{+}$ or $Nrf2^{-}$ host environment at two-time points of tumor progression. In CAAT, tumor growth led to the increased activity of glutathione reductase (GR) in both WT and Nrf2KO mice. On the other hand, GSH levels and glutathione S-transferase (GST) activity decreased in CAAT of Nrf2KO mice when compared to their WT counterparts, irrespective of tumor size. Similar patterns of GSH levels and GR, GST, and glutathione peroxidase (GSH-Px) activity in distant AT highlighted the systemic effects of breast cancer on GSH-related redox response. Furthermore, Nrf2KO mice displayed decreased GST activity in tumor tissue when compared to WT mice, underlining the importance of Nrf2-dependent reprogramming of the tumor microenvironment (TME) in the adaptive redox response of breast cancer. For the first time, these results emphasize not only the importance of Nrf2 in the redox reprogramming of CAAT but also the importance of Nrf2-driven modulations of the TME in the redox response of breast cancer cells.

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OP IV_06

THE EFFECTS OF PHYSICAL ACTIVITY ON GENE-SPECIFIC METHYLATION OF ANTIOXIDANT AND TUMOUR SUPPRESSOR GENES IN POST-SURGERY FEMALE BREAST CANCER PATIENTS UNDERGOING MEDICAL TREATMENT

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The majority of cancer therapies function by triggering the production of reactive oxygen species (ROS) to induce oxidative stress in cancer cells, leading to their death. This frequently causes drug-induced systemic cytotoxicity. Physical activity (PA) has emerged as a comprehensive approach to cancer treatment, yielding beneficial health effects, including the regulation of redox homeostasis. In this study, we examined the influence of an online supervised PA program on the methylation status of specific gene promoters and their corresponding gene expressions/activities, in 3 antioxidants- (SOD1, SOD2, CAT) and 3 breast cancer (BC)-related genes (BRCA1, L3MBTL1, RASSF1A) in women that have been diagnosed with primary BC, undergoing medical treatment. We further examined the genes involved in the methylation and demethylation pathways, the predicted biological pathways and interactions of our exercise-modulated molecules and assessed the functional significance of altered antioxidant markers on aspects such as aerobic capacity/endurance, physical fatigue, and quality of life (QoL). Our results show that PA was able to maintain the levels of SOD activity in blood plasma and significantly increase SOD2 mRNA at the cellular level (≈+77 %), in contrast to the decrease caused by medical treatment. This increase was inversely correlated with SOD2 promoter DNA methylation (≈− 20 %). Furthermore, we found a significant impact of PA on L3MBTL1 promoter methylation (≈− 25 %), also inversely correlated with its mRNA expression (≈+43 %). Finally, PA was able to increase TET1 mRNA levels (≈+15 %) and decrease the expression of DNMT3B mRNA (≈− 28 %). Our findings indicate that the changes in DNA methylation induced by PA impact various signalling pathways and biological processes related to the cellular response to oxidative stress, chromatin organization, antioxidant activity, and DNA/protein binding. These alterations may have a positive effect on clinical outcomes and enhance the response to cancer treatment in BC patients.

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1. Poster Presentations

PP I_A01

ROLE OF MITOCHONDRIAL SODIUM CALCIUM EXCHANGER (NCLX) IN NLRP3 INFLAMMASOME ACTIVATION BY REDOX SIGNAL

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The NLRP3 inflammasome is a cytosolic multiprotein complex that activates the proinflammatory caspase-1, triggering maturation and secretion of proinflammatory cytokines IL-1β and IL-18. Furthermore, it induces pyroptosis, a form of cell death. This response is necessary to initiate the repairing mechanisms, however, an exacerbated activation of the inflammasome can be detrimental. NLRP3 activation is formed by two phases that occur in response to different stimuli. First, the priming stimulus induces the gene expression of its components, while the activation stimulus drives complex assembly and caspase-1 activation. There is a wide range of stimuli that can trigger these phases and, in some of them, mitochondria is involved in the catalytic NLRP3 activation. Previously, we have described a mechanism by which the activity of the mitochondrial sodium/calcium exchanger (NCLX) is required to generate a redox signal, mediated by mitochondrial superoxide production in response to acute hypoxia. We wondered whether this mitochondrial redox signal, driven by NCLX activity, could participate in NLRP3 activation. We have observed that the selective NCLX inhibition affects NLRP3 activation after treatment with lipopolysaccharides (LPS) and ATP, inhibiting the activation signal and not the priming signal. Subsequently, it has been verified that this inhibition prevents the increase in mitochondrial superoxide and peroxide produced by LPS + ATP. To sum up, NCLX may play an important role in NLRP3 activation, although the mechanism is still unclear, so further experiments will be conducted.

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PP I_A02

ARTEMISININ INDUCES LIPID PEROXIDATION IN PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES IN CONCENTRATION- AND MALARIA PARASITE STAGE-DEPENDENT MANNER

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Malaria remained an important and potentially life-threatening infectious disease caused by parasites of the Plasmodium genus. Numerous substances exhibit antimalarial activity, including artemisinin, which has been widely used recently. Artemisinin-based combination therapy (ACT) is recommended for treating *Plasmodium falciparum (P.f.)* malaria worldwide. Additionally, antitumor, immunomodulatory, and several other therapeutically useful properties of artemisinin are currently under investigation. Different mechanisms of action were proposed for dihydroartemisinin (DHA), the active metabolite of artemisinin, such as eliciting oxidative stress in target cells. The objective of this study is to monitor the generation of reactive oxygen species (ROS) and lipid peroxidation product 4-hydroxynonenal (4-HNE) by DHA in *P.f.*-infected human erythrocytes. Examining the kinetics of DHA-elicited ROS generation and protein alkylation with4-HNE throughout the parasite maturation process in the host erythrocyte, highest 4-HNE-adduct levels were observed in young “ring forms” of *P.f.* At low micromolar concentrations, DHA rapidly induced a 2-fold increase of 4-HNE-adducts, which are presumed to be damaging, while no significant increase was elicited by any tested DHA concentration in mature trophozoite stages. Mass spectrometry performed with ring stage proteins revealed extensive

modifications of the P.f. protein cysteine proteinase falcipain-1 by 4-HNE, suggesting the conjugation of crucial P.f. proteins with 4-HNE as cause for DHA-elicited parasite death. In conclusion, significant 4-HNE accumulation was detectable after DHA treatment, though, at concentrations well above pharmacologically effective ranges in malaria treatment, but at concentrations useful for antitumor activity. Human cathepsins B, K, and S, which share similarities with falcipain-1 in the active site, are putative targets of DHA in tumor treatment. Hence, lipid peroxidation with subsequent post-translational modification of functionally relevant proteins by 4-HNE, might be considered as uniform mechanism by which DHA potentiates antimalarials' action in ACT and regulates tumor progression.

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PP I_A03

UTILITY OF SELENOCYANATE TO MODULATE CELLULAR DAMAGE INDUCED BY MYELOPEROXIDASE DURING CHRONIC INFLAMMATION.

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Chronic inflammation is a repetitive cycle of immune cell infiltration and activation that underlies the development of many diseases. Activated immune cells release myeloperoxidase (MPO), which produces hypochlorous acid (HOCl) to kill pathogens. However, excessive production of HOCl during chronic inflammation causes extensive, irreversible host tissue damage. As a result, there is widespread interest developing therapeutic strategies to combat HOCl-induced damage. This study explores the use of selenocyanate (SeCN-) to modulate HOCl-induced cellular damage. SeCN- is both an alternative substrate for MPO and a scavenger of HOCl, which results in formation of hyposelenocyanous acid (HOSeCN). However, the biological reactivity of HOSeCN is poorly characterised. Here we examined the reactivity of HOSeCN with macrophages, which are a key inflammatory cell. Exposure of J774A.1 and THP-1 macrophages to HOSeCN resulted in a dose-dependent loss of viability, assessed by metabolic activity assays and release of lactate dehydrogenase. Flow cytometry with Annexin V and propidium iodide staining revealed that apoptosis was a dominant cell death pathway. Exposure of the macrophages to HOSeCN also resulted in a change in the redox environment within the cells, as evidenced by a loss of intracellular thiols. Interestingly, HOSeCN appeared to be more toxic to macrophages compared to HOCl, in contrast to previous studies with vascular smooth muscle cells. However, unlike the situation with HOCl, preliminary studies suggest that thiol oxidation and cytotoxicity induced by HOSeCN is at least partially reversible. Current studies are examining the mechanisms involved in cytotoxicity and the activation of inflammatory signaling on macrophages exposed to HOSeCN. Together, these results suggest further experiments are necessary to establish the utility of SeCN- supplementation in modulating cell damage in chronic inflammation, as there is some variation in the susceptibility of different cell types to HOSeCN.

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PP I_A04

THE DIAGNOSIS POTENTIAL OF MIRNAS IN SEPSIS AND THEIR RELATION TO OXIDATIVE STRESS

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According to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), sepsis is a "life-threatening organ dysfunction caused by a dysregulated host response to infection". This dysregulation usually entails oxidative stress that further contributes to the profound tissular and organ damage resulting from this syndrome. Some patients develop severe abnormalities at a circulatory, cellular, and metabolic level, linked to a higher risk of mortality, and are diagnosed with septic shock. Furthermore, these patients may present associated pathologies that further aggravate the state and survival probability. All these factors contribute to the heterogeneity of the syndrome, and thus a correct selection of biomarkers is needed for the accurate and fast diagnosis and prognosis prediction. Moreover, the first 6 hours of admission are critical, especially for the selection and administration of the most appropriate treatment. In recent years, the field of epigenetics has aided in the fast and correct stratification of patients depending on measurable biomarkers. Specifically, microRNAs (miRNAs), a type of non-coding RNA, have captured the interest due to being a robust, stable, and fast-measurable biomarker in body fluids. The expression levels of miRNAs have been closely related to oxidative stress and in the control of antioxidant responses, and both processes are dysregulated in sepsis and septic shock. In this work, we aim to establish the circulating miRNA expression signature in sepsis composed of miR-24, miR-122, and miR-146 by analysing patients' plasma using RT-qPCR, which could rapidly classify patients into sepsis or septic shock and help detect adverse side effects, such as disseminated intravascular coagulation. Additionally, we measured carbonylated proteins in plasma samples of the same patients using the OxyBlot technique and found that carbonylated proteins were higher in septic shock patients than in septic and control subjects.

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PP I_A05

CHANGES OF UNCONJUGATED BILIRUBIN LEVELS AND DNA DAMAGE IN HOSPITALISED IN MIDDLE-AGED COVID-19 PATIENTS

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Viral infections are commonly associated with adverse changes in oxidative stress-related pathways, elevated inflammation and increased DNA damage. However, clinical data regarding COVID-19 and alterations of oxidative stress markers is still limited. Thus, this case-control study was designed to investigate whether hospitalised COVID-19 patients differ from healthy age- and sex-matched controls in oxidative stress-related markers, unconjugated bilirubin (UCB), a COVID-19 related Olink inflammatory marker panel and DNA damage. We used HPLC for the analysis of UCB and analysed plasma oxidative stress as well as clinical biomarkers. The Comet assay was applied to investigate oxidative DNA damage using formamidopyrimidine DNA glycosylase (FPG) and H_2O_2 challenge. COVID-19 patients (n=48) displayed significantly higher serum levels of 55 inflammatory proteins ($p<0.001$), including C-reactive protein ($p<0.05$). Interestingly, the levels of UCB were significantly lower in individuals with COVID-19, in particular in middle-aged COVID-19 patients (n=24, mean age 55.7 years; ($p<0.05$) compared to healthy controls. Unexpectedly, several markers of oxidative stress remained unchanged (FRAP, malondialdehyde, $p>0.05$) compared to healthy controls. DNA damage (%-DNA in tail) was age-specifically significantly increased after formamidopyrimidine DNA glycosylase (FPG) treatment in middle-aged ($p<0.05$) but not older COVID-19 patients (n=24, mean age 83.5 years; $p>0.05$). These results suggest an age-associated reduction of UCB levels and an increase of DNA damage in middle-aged hospitalised COVID-19 patients that may result from elevated inflammatory status, but not due to changes in oxidative stress.

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