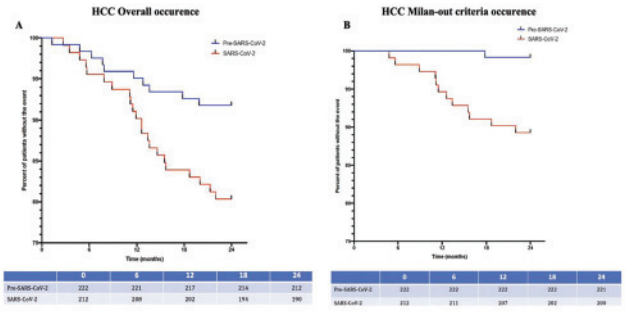
with an increase in body mass index and waist-to-hip ratio (p<0.0001 for all). Low/high-density lipoprotein, glucose, insulin, homeostatic model assessment for insulin resistance, and transaminases worsened at T2 (insulin: p=0.0003, glucose: p=0.0007; p<0.0001 for the others). The non-invasive tools assessments revealed a NAFLD fibrosis score, liver stiffness measurement, and controlled attenuation parameter impairment at T2 (p<0.0001 all). The BIA evidenced an increase in Fat Mass (FM) and a reduction of Free Fat Mass (FFM), Body Cell Mass (BCM), and Extracellular Mass (ECM) at T2 in comparison to the other study time points. During the confinement, the overall HCC and Milan-out staged HCC occurrence revealed HR:2.521, p=0.01, and HR:13.78, p=0.0009 respectively. Contrariwise to the modifications of the biochemical parameters, a significant association between body composition parameters modification during the study evaluations with HCC overall and HCC Milan-out criteria occurrence was highlighted.



The Kaplan-Meier Log-Rank Test analysis comparing the HCC overall (A) and Milan-out criteria at diagnosis (B) occurrence pre- vs during the pandemic period

Conclusions: The confinement-related lifestyle changes impacted NAFLD evolution via body composition modifications, negatively influencing the overall and HCC Milan-out criteria occurrence.

T.03.4

SPHINGOSINES, COUP-TFII AND PANCREATIC CANCER

Polvani S.*[1], Mello T.[1], Ceni E.[1], Guida A.[2], Picariello L.[1], Grappone C.[2], Dragoni G.[3], Lynch E.N.[1], Innocenti T.[1], Tarocchi M.[1], Donati C.[1], Bruni P.[1], Milani S.[1], Galli A.[1]

[1]Università di Firenze, Firenze, Italy, [2]Università di Siena, Siena, Italy, [3]Ospedale Careggi, Firenze, Italy

Background and aim: COUP-TFII (Nuclear Receptor Subfamily 2 Group F Member 2, NR2F2) is an orphan nuclear receptor transcription factor with pleiotropic effects. In pancreatic ductal carcinoma (PDAC), COUP-TFII is linked to a poorer outcome; its higher expression correlates with a reduced survival, epithelial to mesenchymal transition (EMT), and altered invasiveness and stemness. Sphingolipids, such D-erythro-sphingosine and 1-deoxy-sphingosine, may modulate its transcriptional activity; moreover, COUP-TFII expression is associated with a perturbation of the phospholipid's pathway. Sphingosine 1-phosphate (S1P), an autocrine/paracrine cell signaling molecule produced from sphingosine by two sphingosine kinases (SPHK1 and SPHK2), may worsen the progression of several cancers. Nonetheless, the knowledge of sphingosine pathway role in PDAC is limited and its connection with COUP-TFII has never been investigated, hence the aim of this work is to evaluate if a relationship exists between COUP-TFII activity and expression and sphingosine signaling.
Materials and methods: Data mining for the expression of S1P pathway-related genes (SPHK, SPHK2 and SPL) in primary samples was performed with cBioPortal. SPHK2, SPHK1 and SPL survival analysis were conducted on cBioPortal. mRNAs expression in human PDAC cells (PANC-1, MiaPACA2, CAPAN-2, BxPC3, PL-45 and Su.86.86) and in the immortalized ductal cell line hTERT-HPNE were evaluated by qPCR and the ddCt method. β2M and GAPDH were used as housekeeping genes. Proliferation and cell cycle of cancer cells treated with inhibitors for SPHK1 (PF-543) or SPHK2 (ABC 294640) were measured with the appropriate kits on a Muse cell analyzer.
Results: cBioportal analysis suggests that high SPHK2 and low SPL and SPHK1 expression are associated with higher overall survival. Interestingly, in primary PDAC, when SPHK2 expression is at the highest COUP-TFII is at the lowest. However, immortalized cancer cells have higher SPHK2 and lower SPHK1 and SPL compared to the normal ductal cell hTERT-HPNE. ABC 294640 increases cell survival/proliferation of starved cells stimulated with EGF, whereas PF-543 has the opposite effect. Inhibition of either of SPHKs alters the expression of COUP-TFII and of its down targets, whereas overexpression of the nuclear receptor affects the sphingosine kinases.
Conclusions: Our results suggest that the sphingosine signaling pathway and COUP-TFII are intertwined, and they could act together in the modulation of PDAC progression.

T.03.5

EFFECT OF ENVIRONMENTAL BISPHENOL A EXPOSURE ON TRAINED IMMUNITY-RELATED PATHWAYS IN NON-ALCOHOLIC FATTY LIVER DISEASE: A PRELIMINARY OBSERVATION

Ventriglia L.*[1], Scognamiglio F.[1], Romeo M.[1], Cipullo M.[1], Moggio M.[2], Niosi M.[1], Diano N.[2], Dallio M.[1], Federico A.[1]

[1]Department of Precision Medicine, Hepatogastroenterology Unit, University of Campania "Luigi Vanvitelli", Naples, Italy, [2]Department of Experimental Medicine, University of Campania Luigi Vanvitelli,, Naples, Italy

Background and aim: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver illness in Western countries. Bisphenol A (BPA) is a well-known endocrine disrupter, widely produced worldwide and cumulated in human body. Trained Immunity (TI) represents a novel concept of the immunological response involving the innate immune cells, triggered by a second antigenic contact, gaining a long-term reversible pro-inflammatory phenotype. Current knowledge suggests BPA implication in the immunometabolic switch which could result in a different immune reactivity. We aimed to explore whether BPA influence TI reactivity in NASH patients due to a possible hyperelicitation of pro-inflammatory pathways.
Materials and methods: Serum samples of healthy individuals (n.30) and histologically proven NASH patients (n. 30) were subjected to liquid-liquid extraction of bisphenol A with methanol, then to solid-phase extraction cartridge for clean-up and concentration. The analysis of sample extracts was carried out by HPLC system coupled to triple quadrupole mass spectrometer. In parallel, for each subjects a peripheral venous blood sample was collected (20 mL). Consequently, monocytes were isolated through a density gradient centrifugation and then stimulated with BPA for 24 h (1 nM, 10 nM, 20 nM) and lipopolysaccharide (LPS) (10 ng/mL) for 24 h (at day 0 and day 6 respectively). In order to assess the pro-inflammatory [tumor necrosis factor (TNF)-α, interleukin (IL)-6] and anti-inflammatory response [IL-10] ELISA assays were performed.
Results: The mean values of BPA quantification in serum samples of healthy individuals was 0.1398±0.0495 ng/mL in comparison to NASH patients 7.79±2.34 (P<0.0001). The induction of TI mediated by BPA (1 nM, 10, nM and 20 nM) emerged higher in NASH patients compared to healthy individuals (p<0.05). The hyperelicitation of TI mechanisms in monocytes has been expressed by the high levels of pro-inflammatory cytokines (TNF-α and IL-6) in NASH patients respect to healthy ones in each concentration chosen for the stimulation. Additionally, our data also showed an increased