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95th National Congress of the Italian Society for Experimental Biology

Trieste, Italy, 12-15 April 2023

ABSTRACT BOOK

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TABLE OF CONTENTS

woods *e.g.*, walnut, mahogany and ebony but it can also affect the soft woods *e.g.*, conifers. The nickname "woodworm" is related to a long-term life cycle phase when the soft, white, moisture-loving, vermiform larvae dig tunnels in the wood, degrading cellulose/ starch, leaving deposits of wood fibres and faecal pellets (rosume). The main track of their presence is evident only after the reddish-brown adults flick from the wood, through the characteristic exit holes (1-1.5 mm in diameter). This case study focuses on the damage caused by woodworms on old wooden statue located in the Augustinian Novacella Abbey (Neustift Abbey, Vahrn), one of the most important monasteries in the South Tyrol area.The *in situ* investigative protocol to find tracks of xylophagous insects in the artworks was based on: (a) visual survey to assess the presence/absence of exit holes, fine sawdust and adults/larvae/pupae on the wood surface or exposed tunnels with the aid of a magnifying glass; (b) photographs of the wooden material and specimens; (c) sampling ; (d) worklab for Light Microscopy identification of larvae/adults/pupae on the basis of diagnostic morphotraits. The last step is the planning and application of standard protocols of pest control in the focused scenario. *A. punctatum* is the main beetle pest of the wooden goods heritage in Italy. Applied research is in progress to standardize sustainable approaches for a green pest control.

EFFICACY OF THE SORAFENIB TREATMENT IN A 3D HEPATIC CANCER MODEL BY COMPARING STATIC AND DYNAMIC CULTURE CONDITIONS

Hélia FERNANDES[1], Viviana VALERI[1], Cristina DEGRASSI[1]

[1]MTTlab Srl, Trieste, Italy

Drug development is a long and highly expensive process. During the preclinical research phase both *in vitro* and *in vivo* models are used but with some limitations. The classical human *in vitro* setup fails to recapitulate the complexity of systemic biology, while animal models do not exactly reproduce human biology. In the last years, ethical concerns about the wide use of animals for research purposes have emerged. This promoted the development of *in vitro* models that could better mimic the complexity of tissues and biological systems. 3D cell structures developed in the last years represent very promising models for both healthy and pathological setups. For the study of tumors, the use of 3D spheroids or organoids allows the establishment of cell-to-cell interactions, in a more realistic tumor microarchitecture when compared to the 2D tumor cell cultures. We developed a reusable platform to generate thousands of 3D spheroids, from the human hepatocellular carcinoma HepG2 cell line, in a faster way. The spheroids are homogenous in shape and size and remain viable. Moreover, we can easily retrieve the 3D spheroids from the platform and use them for further experiments. We are currently developing a system to test in physiological-like conditions the cytotoxicity of Sorafenib, a reference drug used in the treatment of hepatic cancer. The spheroids are inserted in a commercially available bioreactor (MIVO^{R,} React4Life) in which we can reproduce the systemic administration of the compound by using a dynamic flow. The bioreactor has already been used to show the efficacy of the Cisplatin treatment in an ovary cancer cell model with a prediction that closely matches the *in vivo* results, requiring a much shorter experimental time [1]. Our preliminary results show a difference in the cytotoxic effect on the Sorafenib-treated HepG2 spheroids by comparing static and dynamic treatment conditions. This approach can thus become a reliable method to speed up the preclinical research phases and contribute to the reduction of the use of animals.

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ADIPOSE DERIVED STEM CELL BEHAVIOR IS INFLUENCED BY MCF-7 EXHAUSTED MEDIUM

Giuseppe GARRONI[1], Sara CRUCIANI[1], Renzo PALA[1], Margherita MAIOLI[1,2]

[1]Department of Biomedical Sciences, University of Sassari, Sassari; [2]Center for developmental biology and reprogramming CEDEBIOR, Department of Biomedical Sciences, University of Sassari, Sassari, Italy

Stem cells are an excellent tool for regenerative medicine. They can be isolated from various tissues, including adipose tissue. Adipose tissue is used in reconstructive medicine, as after mastectomy, to restore a natural appearance following surgery. Adipose derived stem cells (ADSCs) are able to differentiate into different cell lines after different type of stimulation. The cellular environment is able to influence the fate of stem cells residing in the tissue. In this work, we investigated how an exhausted breast cancer cell line (MCF-7) medium is able to influence stem cell differentiation. Cells were exposed to an exhausted medium harvested at different time points. After treatments, we evaluated the expression of stemness genes, adipogenic and osteogenic differentiation-related genes. To confirm the gene expression data, the oil red and alizarin red colorimetric assay was performed. The data reported here demonstrate that stem cells exposed to the differentiation medium maintain a stemness phenotype with high proliferation rate.

CELL CYCLE BLOCK BY p53 ACTIVATION REDUCES SARS-COV-2 RELEASE IN INFECTED ALVEOLAR BASAL EPITHELIAL A549-hACE2 CELLS

Giada LODI[1], Valentina GENTILI[2], Fabio CASCIANO[3,6], Arianna ROMANI[1,6], Giorgio ZAULI[4], Paola SECCHIERO[3,6], Enrico ZAULI[3], Carolina SIMIONI[5], Silvia BELTRAMI[2], Mercedes FERNANDEZ[2], Roberta RIZZO[2], Rebecca VOLTAN[1,6]

[1]Department of Environmental and Prevention Sciences, University of Ferrara, Italy; [2]Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Italy; [3]Department of Translational Medicine, University of Ferrara, Italy; [4]Research Department, King Khaled Eye Specialistic Hospital, Riyadh, Saudi Arabia; [5]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy, [6]LTTA Centre, University of Ferrara, Italy

The two last decades have shown great sanitary emergencies due to the pandemic diffusion of SARS-CoV-2 that has presented a new scientific challenge for the search of effective therapies against infection, replication and spreading. Among the intracellular targets of the virus, p53 is one of the targets that plays an important role both in the mechanisms of innate immunity as well as in the control of the cell cycle and other pathways that regulate cell replication, damage repair, apoptosis and metabolism. SARS-CoV viruses adopt several strategies to silence p53, including the stabilization of its inhibitor, MDM2,

and the interference with its transcriptional activity, indicating that p53 has a central role in controlling its proliferation in the host. For these reasons, the aim of the project was to evaluate a new approach against the virus, by using MDM2 inhibitors to effectively raise p53 levels and activate p53-dependent pathways including cell cycle inhibition. Experiments setting was done in the alveolar basal epithelial cell line A549-hACE2 expressing $TP53^{wild\text{-}type}$ and the SARS-CoV2 receptor ACE2. Cells were treated with several concentration of Nutlin-3 or RG-7112 at the time points of 24 and 72 hours post treatment for the instauration of a cell cycle block steady-state condition before and during SARS-CoV-2 infection, and for the evaluation of p53 activation and impact on virus release and related innate immune events.The results of the project suggest that Nutlin-3, as well as RG-7112, significantly reduced SARS-CoV-2 replication in A549-ACE2 cells and promoted a complete inhibition of IL-6 expression, associated with inhibition of NF-kB and interferon-lambda, important mediators of inflammation. These data indicate that p53 represents an efficient target for new therapies against the virus and that MDM2 inhibitors can be a realistic therapeutic option.

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IMPACT OF PROGRAMMED CELL DEATH PROTEIN 1 VARIANT ON LIVER TRANSCRIPTOME AND NONALCOHOLIC FATTY LIVER DISEASE PROGRESSION

Giulia LUPO[1], Rossella ZITO[1], Ayesha JAVED[1], Gabriele DI MARIA[1], Claudia LA MANTIA[1], Daniela CARLISI[2], Stefania GRIMAUDO[1], Rosaria Maria PIPITONE[1]

[1]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo, Palermo; [2]Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, Palermo, Italy

Nonalcoholic fatty liver disease (NAFLD) accounts for a growing proportion of cases of chronic liver disease, liver decompensation and hepatocellular carcinoma (HCC), and it represents an emerging indication for liver transplantation. Evidences support a role of immune response in promoting nonalcoholic steatohepatitis (NASH), the inflammatory progressive form of NAFLD, leading to fibrogenesis and development of HCC. The programmed cell death protein 1 (PD1) is an immune checkpoint surface receptor, which plays a prominent role in maintaining the immune tolerance and, moreover, it is involved in immune escape from cancer. Programmed cell death protein 1/programmed death-ligand 1 (PD-1/PDL-1) axis has been reported to modulate liver inflammation and progression to HCC in patients with NAFLD. Recent data, in experimental models and in clinical samples, suggest that PD1 is expressed by a subset of CD8+ cells with exhausted phenotype, which accumulate during NASH inducing a non-antigen restricted killing of steatosis hepatocytes. The exhausted T cells facilitate the development of inflammation leading to disease progression and promoting hepatic carcinogenesis. The aim of our study was to evaluate, in a wide multicentric cohort, the impact of *PDCD1* gene variants, rs13023138 G>C, on NASH patients, who have a potential to develop HCC. Moreover, hepatic transcriptome was examined by RNASeq in a subset of patients. Transcriptomic and deconvolution analysis were performed to identify biological pathways modulated by the risk allele. Our results reported that the frequency distribution of PDCD1 rs13023138 CC, CG and GG genotypes was 38.5%, 45% and 16.5%, respectively. Genetic frequencies fitted Hardy–Weinberg equilibrium. Moreover, rs13023138 G allele was linked to higher hepatic representation of M1 macrophages, together with the upregulation of pathways related to inflammation and higher expression of C-X-C Motif Chemokine Receptor 6 (CXCR6). In our cohort, the *PDCD1 rs13023138* G allele was independently associated with severe steatosis, NASH, advanced fibrosis and HCC, suggesting a role of this polymorphism in the monitoring of NAFLD progression and HCC development.

MORPHO-FUNCTIONAL MODIFICATIONS OF GUT BARRIER PROPERTIES INDUCED BY SURFACE LAYER PROTEINS (S-LAYER) FROM *Lactobacillus helveticus* ATCC® 15009™ IN A CO-CULTURE OF CACO2/HT-29 CELLS AS A MODEL OF HUMAN INTESTINAL EPITHELIUM

Federica PIAZZALUNGA[1], Paola BENDINELLI[1], Milena BRASCA[2], Tiziana SILVETTI[2], Ivano DE NONI[3], Stefano CATTANEO[3], Elena DONETTI[1], Anita FERRARETTO[1]

[1]Department of Biomedical Sciences for Health, University of Milan; [2]Institute of Sciences of Food Production, National Research Council (CNR-ISPA), Milan; [3]Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy

Healthy gut barrier (GB) can be improved by specific probiotic strains, even if their reliability and safety concerns in clinical practice are largely discussed. Paraprobiotics and postbiotics can represent a valid alternative due to their intrinsic higher stability and preservation in food matrices. Among probiotic species, Lactobacilli constitute an important group of microorganisms able to stimulate host's immune system and act as an effective therapeutic alternative for the treatment of gut inflammation, obesity, and chronic degenerative diseases. The present study investigated the effects of surface-layer proteins (S-L) of the dairy strain *Lactobacillus helveticus* ATCC® 15009™ (*Lh* ATCC® 15009™) on the morpho-functional modulation of GB in comparison to live or heat killed *Lh* ATCC® 15009™ in a Caco-2/HT-29 70/30 co-culture cells. Live or heat-killed *Lh* ATCC® 15009™ (100 CFU/cell and 1000 CFU/cell) negatively affect transepithelial electrical resistance (TEER) and paracellular permeability, resulting in an altered distribution of tight junction (TJ) and protein Claudin-1, stained by immunofluorescence (IF). Conversely, the addition of S-L, in amounts present into the doses of *Lh* ATCC® 15009™ administered to cells, improves TEER, and decreases permeability in physiological conditions only when basal TEER registered in co-cultures established by Caco-2 and HT-29 parental cell lines with at least 40 and 21 sub cultivation passages respectively is minor than 50 ohm*cm^2. This experimental condition may suggest the presence of a physiologically leaky gut as it occurs in old people. Transmission electron microscopy (TEM) and IF analyses suggest that S-L induces a structural TJ rearrangement and desmosomes' formation and stability. S-L is also able to restore TEER and permeability of GB in the presence of lipopolysaccharide (LPS), but not of pro-inflammatory cytokines (TNF-α plus IFN-γ). IF analysis shows an increase in Claudin-1 staining when LPS and S-L were co - administered, suggesting that the downstream Toll-Like Receptor- mediated signaling (TLR4 for LPS, TLR2 for S-L) may result in junctional apparatus remodeling, such as increased desmosomes' protein complexes transcription or TJ protein phosphorylation and redistribution. In addition, S-L can counteract the reduction of alkaline phosphatase detoxification activity and the enhancement of pro-inflammatory interleukin-8 (IL-8) release both induced by LPS. Altogether, these data obtained in a model of