

Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale



**93rd National Congress of the
Italian Society of Experimental Biology**

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ABSTRACT BOOK

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in a 3D model of organotypic cultures of normal human skin standardized in our lab, strictly mimicking the physiological condition. Bioptic skin fragments obtained after aesthetic surgery of healthy women (n=7) were cultured in a Transwell system with either IL-4 (50 ng/ml) or IL-13 (50 ng/ml) and harvested after 24 and 48 hours, with parallel control groups. All samples were processed for light microscopy (LM), immunofluorescence (IF), and transmission electron microscopy (TEM) analysis. As biomarkers of terminal differentiation, keratin (K)14 and K10, respectively expressed in the basal and the suprabasal layers, and filaggrin, typically found in the granular layer, were analysed. K16 and K17, absent in normal skin, were assessed as skin alarmins. The molecular composition of tight junction (TJs) encompassed claudin-1 and zonula occludens (ZO)-1 expression. Quantitative analysis was performed for i) Langerhans cells (LCs) on blue toluidine-stained semithin sections, ii) keratin immunostaining after indirect IF, and iii) intercellular spaces by TEM observations. Both cytokines induced a time-dependent increase of LCs number, a K14 immunostaining weakening, and K17 expression in scattered suprabasal keratinocytes. At 24 hours, IL-13 elicited the dilation of intercellular spaces throughout the entire epidermal compartment and the restriction of K10 immunoreactivity from the medium spinous layer upwards. K16 induction and discontinuity of filaggrin immunostaining were always more evident in IL-13 than in IL-4 groups. Regarding TJs, the two cytokines inhibited similarly claudin-1 expression at both time points. Conversely, ZO-1 distribution was more affected by IL-13 than by IL-4. In our experimental conditions, IL-13 initially triggers the modulation of keratin expression and the molecular composition of TJs. Later, IL-4 sustains the existing pro-inflammatory milieu, with weaker effects than IL-13. Synergic or additional actions between IL-4 and IL-13 in AD progression will be evaluated.

SICILIAN MANGO PEEL INDUCES CELLULAR STRESS ACCOMPANIED TO MITOCHONDRIAL DYSFUNCTION IN COLON CANCER CELLS

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Currently, cellular stresses as the oxidative, metabolic and genotoxic stress are considered the cause of many different human pathologies as neurodegenerative diseases (e.g., Alzheimer's, Parkinson's, amyotrophic lateral sclerosis), alcoholic liver disease, chronic obstructive pulmonary disease, and also cancer. Although the role of cellular stress has been largely debated in cancer, nowadays some therapies aim to target the intracellular pro-oxidant/anti-oxidant balance triggering the tumor commitment to cell death. Therefore, it has become more necessary an improved understanding of cancer response to cellular stress that could be advantageous to develop cancer tailored therapies. In this scenario, the present study shows how extracts of some fractions of Sicilian mango, a tropical fruit rich in phytochemicals with nutraceutical properties, are able to affect the cell viability of three colon cancer cell lines (HT29, Caco2 and HCT116) inducing cellular stress. By using hydro-alcoholic extracts of three different portions of the fruit (peel, pulp and kernel), we observed that mango peel extract (MPE) is the most effective in reducing cell viability,

causing a remarkable LDH release and the death of all three cancer cells. The effect was accompanied by mitochondrial injury, dissipation of mitochondrial potential membrane and decrease in the level of proteins localized in the mitochondrial membrane such as voltage-dependent anion-selective channel (VDAC), mitofilin, and some members of Bcl-2 family proteins (Mcl-1, Bcl-2 and Bcl-XL). All these effects were accompanied by redox balance changes and upregulation in MnSOD, a mitochondrial scavenger enzyme able to modulate the cellular response against oxidative damage. The analysis of the effects exerted by the different phytochemicals present in MPE allowed to identify those molecules responsible for the observed anticancer effects sustaining their future employment as chemopreventive or therapeutic agents.

A NOVEL HYPOTETICAL MECHANISM OF ERYTHROCYTE SENESCENCE?

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The erythrocyte senescence mechanism and its subsequent spleen removal remain still unknown. The view that the aging of the red cell and its removal from the circulation result from a progressive series of events during the 120 days of its lifespan appears to be the most consistent with the available data. One of the best described phenomena that relate to erythrocyte senescence during the erythrocyte lifespan is the Gardos effect, in which red blood cells (RBCs) loss through this channel the KCl and gain NaCl and this lead the cells to dehydration, as well are involved the oxidative stress due to the reactive oxygen species (ROS) that can damage the RBCs function, vesiculation of the erythrocyte membrane, the exposure of the phosphatidylserine (PS) as a death signal for the RBCs. Taking into account all these changes and several other that occur and lead red blood cells to senescence, we proposed a new mechanism that could lead to senescence and its spleen removal. Our findings indicate that hemichromes possess a strong affinity for band 3 cytoplasmic domain and, following their binding, lead to band 3 oxidation and clusterization. Those band 3 clusters show increased affinity for NABs which activate complement and finally trigger the phagocytosis of altered RBC. Persistent tyrosine phosphorylation of Band 3 protein produces extensive membrane destabilization leading to the loss of vesicles containing hemichromes. Understanding this mechanism should provide insight into the critical membrane alterations in hereditary hematology disorders and malaria that are characterized by tyrosine hyperphosphorylation of band 3.

BIOACTIVE TRITERPENES OF *Protium heptaphyllum* GUM RESIN EXTRACT DISPLAYED CHOLESTEROL-LOWERING POTENTIAL

Giuseppe MANNINO¹, Piera IOVINO³, Antonino LAURIA¹, Tullio GENOVA², Alberto ASTEGGIANO^{3,4}, Monica NOTARBARTOLO¹, Alessandra PORCU⁵, Graziella SERIO¹, Giorgia CHINIGÒ², Andrea OCCHIPINTI⁵, Andrea CAPUZZO⁵, Claudio MEDANA⁴, Luca MUNARON², Carla GENTILE^{1,*}

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