SS I_05

D-ALANINE, A METABOLITE OF GUT MICROBIOTA, INHIBITS INTESTINAL INFLAMMATION BY SUPPRESSING THE ACTIVATION OF MACROPHAGE

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Free D-amino acids, which have different functions from L-amino acids and produced by gut microbiota, have recently been discovered in various tissues. However, studies on the potential interactions between intestinal inflammation and D-amino acids are limited. We examined the inhibitory effects of D-alanine (D-Ala) on the pathogenesis of intestinal inflammation. Serum D-Ala levels were significantly lower in patients with ulcerative colitis (UC) than in healthy volunteers. The ratio of D-Ala to L-Ala was significantly lower in patients with UC in clinical activity than in healthy volunteers. Dextran sulfate sodium-treated mice had significantly lower plasma D-Ala levels than control mice. D-Ala-treated mice had significantly lower disease activity index than control mice. *IFN-γ*, *IL-12p35*, *IL-17A*, and *IL-23p19* mRNA expression levels were significantly lower in D-Ala-administered mice than in control mice. D-Ala suppressed naïve T cell differentiation into Th1 cells *in vitro* and inhibited the production of IL-12p35 and IL-23p19 in bone marrow-derived macrophages. Our results suggest that D-Ala prevents dextran sulfate sodium-induced colitis in mice and suppresses IL-12p35 and IL-23p19 production in macrophages.

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SS I_06

PROTECTIVE EFFECTS OF SOME COX-2-DERIVED BIOACTIVE LIPID MEDIATORS ON EXPERIMENTALLY INDUCED COLITIS AND INTESTINAL CARCINOGENESIS

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While cyclooxygenase-2 (COX-2) is a key enzyme involved in mediating proinflammatory signaling, some of its products have anti-inflammatory and pro-resolving activities. Examples are 15-deoxy-^{12,14}-prostagandin J_2 (15d-PGJ_2), D- and E-series resolvins, 17-oxo-docosahexaenoic acid, and 15-ketoprostaglandin E_2. Our previous study demonstrated the involvement of locally produced 15d-PGJ_2 in the resolution of intestinal inflammatory responses. Thus, pharmacologic inhibition of 15d-PGJ_2 biosynthesis hampered resolution of inflammation in the colonic mucosa of mice treated with dextran sulfate sodium (DSS). In contrast, intraperitoneal injection of 15d-PGJ_2 accelerated the resolution of experimentally induced murine colitis. Moreover, mice treated with exogenous 15d-PGJ_2 exhibited the significantly reduced proportion of macrophages expressing the proinflammatory cytokine, interleukin-6 (IL-6). 15d-PGJ_2 activates nuclear factor erythroid-2-related factor-2 (Nrf2) through covalent interaction with Keap1, an inhibitory protein that sequesters Nrf2 in the cytoplasm. We have found that Nrf2-mediated expression of heme oxygenase-1 contributes to the resolution of DSS-induced colitis by stimulating macrophage polarization. Resolvin D1 (RvD1) is a prototypic specialized pro-resolving mediator formed from docosahexaenoic acid by COX-2 in the presence of aspirin during the resolution phase of inflammation. We have reported a marked reduction in the plasma levels of RvD1 in the colorectal cancer patients and mice harboring the inflammation-associated colon tumor. Administration of RvD1 intraperitoneally attenuated DSS-induced colitis and azoxymethane plus DSS-induced colorectal carcinogenesis, which were associated with IL-6 and IL-6-mediated chromosomal instability. Phagocytic removal of dying/dead cells including apoptotic neutrophils, termed 'efferocytosis', prevents the tissues surrounding the inflamed site from being exposed to the toxic contents of lytic cells. In a murine peritonitis model, intraperitoneal administration of RvD1 abolished the zymosan A-induced TNF-α production, thereby stimulating efferocytosis. Further studies will be necessary to evaluate the tumor suppressive functions of endogenous bioactive lipid molecules and underlying molecular mechanisms.

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SS I_07

LONG CHAIN METABOLITES OF VITAMIN E AND THEIR POTENTIAL IN INFLAMMATION RESOLUTION

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Identification of metabolites occurred during the hepatic metabolism of Vitamin E opens a novel area of interest. Garcinoic acid (GA) and α-13′-carboxychromanol (α-13′-COOH), a natural derivative of δ-tocotrienol and long chaing metabolite of α-tocopherol, are developing area of interest in regulating various process, including inflammation. Clearance of apoptotic cells in inflammatory area through phagocytosis (efferocytosis) is one of crucial process that prevents the transition to postapoptotic necrosis. As efferocytosis capacity of macrophages decreased in inflammation, our research focus in the field of potential molecules promoting efferocytosis activity, including the Vitamin E and their metabolites. Although the various vitamin E metabolites has shown to enhance anti-inflammatory mechanisms, there is little information about their involvement in inflammation resolution, especially efferocytosis. We evaluated the impact of α-13'-COOH and GA, with a special focus on unravelling inflammatory status and efferocytosis activity. Both *in vitro* and *in vivo* efferocytosis were observed using bone marrow derived macrophage cells and disease models, respectively. Additionally, various cytokines and lipid-derived mediators of inflammation were assessed to further explore the role of metabolites in resolution and tissue regeneration. Our findings indicate the capacity of α-13'-COOH and GA in enhancing inflammation resolution and support their potential in promoting efferocytosis activity.

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SS I_YIP_01

REVISITING THE ROLE OF H_2O_2 IN NRF2 ACTIVATION USING AN OXYGEN-INDEPENDENT BIOSENSOR

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NRF2 (Nuclear factor erythroid 2-related factor 2) is a crucial transcription factor that governs the expression of genes involved in oxidative stress response, cellular defense mechanisms, and energy metabolism. Impaired NRF2 function has been implicated in aging and various age-related diseases, including neurodegeneration. Despite its significance, the study of Nrf2 in living model systems has been hindered by the lack of suitable tools, leading to discrepancies in the literature. One unresolved question is whether and to what extent H_2O_2, a well-known reactive oxygen species (ROS), activates Nrf2. To address this, fluorescent protein (FP)-based biosensors are promising, yet the oxygen dependency of chromophore formation in prevalent FPs poses challenges under hypoxic conditions, potentially yielding inaccurate results. To overcome this limitation, we developed an oxygen-insensitive Nrf2 biosensor reporter based on miniGFP FP, which incorporates flavin to fluoresce, allowing us to monitor Nrf2 behavior regardless of pericellular oxygen levels. Our preliminary findings indicate that the administration of H_2O_2 whether exogenous (bolus; 1-300 uM, up to 24 hours) or endogenous (using mDAAO enzyme; 0.1-50 mM D-alanine, up to 24 hours) is insufficient to stimulate Nrf2 in human cerebrovascular endothelial cells (hCMEC/D3). More interestingly, treatment with Auranofin (0.1-1 uM, up to 24 hours), a TrxR inhibitor, led to significant increases in Nrf2 levels under normal cell culture oxygen conditions (18% O_2). However, when replicating the

experiment in cells adapted to normoxia (5% O_2), no Nrf2 activation was observed. Collectively, our findings underscore two striking facts. Firstly, for H_2O_2 to induce Nrf2 activation, abating the antioxidant machinery is essential. Secondly, in comprehensively studying redox biology phenomena, accounting for oxygen level is indispensable; otherwise, the translational relevance of results to clinical settings may be compromised.

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SS I_YIP_02

GARCINOIC ACID AND α-13′-COOH MEDIATED MODULATION OF ERASTIN-INDUCED FERROPTOSIS THROUGH NRF2/HO-1 SIGNALING IN HEPATOCYTES

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Ferroptosis is a distinct form of intracellular iron-dependent cell death characterized by reactive oxygen species (ROS) induced lipid peroxidation in excessive amounts. Erastin, widely-used small molecule in activating ferroptosis through the inhibition of cysteine import, results in a deficiency of glutathione, which causes glutathione peroxidase 4 (GPX4), a crucial phospholipid peroxidase, to malfunction. The Nuclear factor erythroid2-related factor 2 (Nrf2)/Heme oxygenase 1 (HO-1) signaling maintains cellular homeostasis under stress and is involved in ferroptosis. Vitamin E is a well-known signal molecule that naturally exists in many foods and has been shown to possess numerous health benefits. Recent research indicates that α-tocopherol, a significant constituent of vitamin E, might decrease lipid peroxides and prevent ferroptosis. However, it remains unclear whether Garcinoic acid (GA), a natural derivative of delta-tocotrienol, and α-13'-carboxychromanol (α-13'-COOH), a long-chain metabolite of vitamin E, can also prevent ferroptosis. Herein, we induced ferroptotic cell death in AML12 mouse hepatocyte cells using erastin and then evaluated the impact of GA and α-13'-COOH, either solo or in combination. We also investigated the therapeutic effect of GA and α-13'-COOH on the NRF2/HO-1 pathway using NRF2 siRNA transfection and HO-1 inhibitor, Zinc Protoporphyrin-IX (Znpp-IX). Our findings provide novel insights into the role of GA and α-13'-COOH in preventing erastin-induced ferroptosis and the potential to stimulate further investigation into the treatment of diseases caused by ferroptosis.

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SS I_YIP_03

CHARACTERIZATION OF EVOLUTIONARY DYNAMICS FOR DRUG RESISTANCE IN A 3D CELL CULTURE SYSTEM USING CELLULAR BARCODING TECHNOLOGY

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One of the main challenges in the treatment of cancer is the complex evolutionary processes regulating drug resistance. Acquired drug resistance is attributed to the selection of pre-existing intrinsically resistant subclones, or it can be de novo, and cellular barcoding technology based on next-generation sequencing can be used to track clones to better understand these mechanisms. Several studies point out that 3D cell culture is a useful experimental model system to effectively mimic and investigate drug resistance and tumor physiology. Combining cellular barcoding technology with clinically relevant model system, namely 3D spheroids, to better mimic drug resistance has been a gap so far. Therefore, we sought to generate dabrafenib- and irinotecan-resistant derivatives of barcoded 3D spheroids to assess selection-induced clonal dynamics and identify genomic determinants in this model system. We found that pre-existing and de novo resistant barcodes promote drug resistance caused by dabrafenib and irinotecan in 3D-HT-29 and 3D-HCT-116 spheroids, respectively, suggesting the existence of polyclonal drug resistance in this system. In addition, whole-exome sequencing analysis on dabrafenib and irinotecan resistance in 3D-HT-29 and 3D-HCT-116 spheroids showed chromosomal gains and mutations compared to their drug-sensitive counterparts. Finally, we demonstrate that multiple drug resistance also mediates resistance to dabrafenib and irinotecan by detecting overexpression of the drug efflux pumps, ABCB1 and ABCG2, in our spheroid model system. In summary, in this study, we provide for the first time an assessment of evolutionary dynamics in drug-resistant spheroids using cellular barcoding technology and the underlying genomic determinants.

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SS I_YIP_04

ADVANCED LIPIDOMICS STRATEGIES TO EXPLORE HEPATIC LIPOTOXICITY AND ITS TREATMENT

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Recent advancements in lipidomics, a post-genomics discipline mainly based on mass spectrometry technology and methods, are now offering unprecedent opportunities to study tissue lipids at different levels of precision and for different applications, including the characterization of the cellular lipidomes, the identification of biomarkers and mechanisms of pathological conditions, and drug discovery. Lipidomics methods have demonstrated great potential in the study of lipid metabolism disorders and lipotoxicity states associated with obesity and deriving from the ectopic deposition of fat in different tissues; these include hepatic lipotoxicity, a multifactorial process of damage of the liver cell triggered by the excess and metabolic effects of cellular lipids, with key pathogenic role in non-alcoholic/metabolic fatty liver disease and steatohepatitis (MAFLD and MASH, respectively). A few lipidomics strategies are now available to study these aspects, including untargeted, targeted and dynamic analysis (or fluxomics) methods; these will be presented in this paper together with examples of their application in the study of mechanistic aspects and treatment of human liver cell lipotoxicity with vitamin E and melatonin. This latter was identified as an effective cytoprotective agent with effects on the cellular lipidome useful in preventing free fatty acid and cadmium-induced lipotoxicity in human liver cells. Lipidomics data also revealed mechanistic insights on the therapeutic role of these agents behind their primary role as antioxidant and hormonal compounds, respectively, highlighting the great potential of this omics technique in drug discovery and development.

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SS I_YIP_05

MESSAGE MISSION: UNDERSTANDING THE IMMUNE SYSTEM AND CANCER CONNECTION IN MICROGRAVITY

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The "Microgravity Associated Genetics Science Mission" (MESSAGE), conducted in Axiom-3 and supported by the Turkish Space Agency and TUBITAK UZAY (SPACE TECHNOLOGIES RESEARCH INSTITUTE) in January 2024, represents a pioneering endeavor in the exploration of genetic responses to microgravity. Üsküdar University TRGENMER laboratory aimed to analyze the transcriptome profile ¨ (pro-inflammatory profile, clock-gene expressions, anxiety and major depression associated gene expression, neurodegeneration associated gene and miRNA expression, anti-aging gene expression including telomere, SIRT family,