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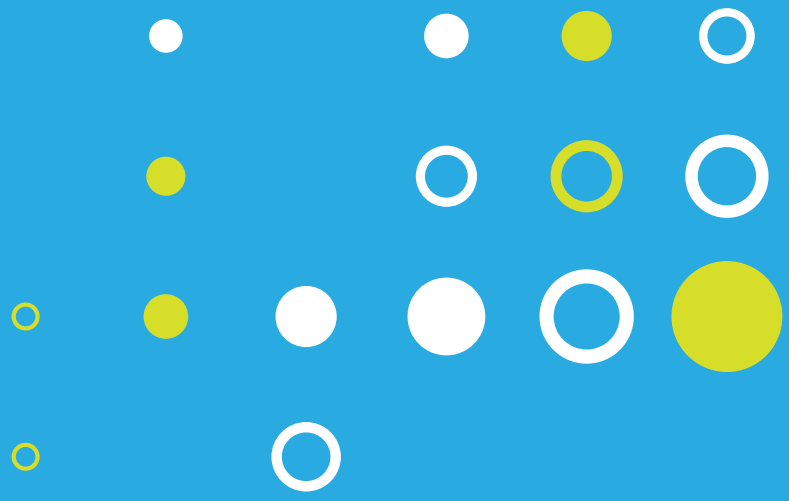

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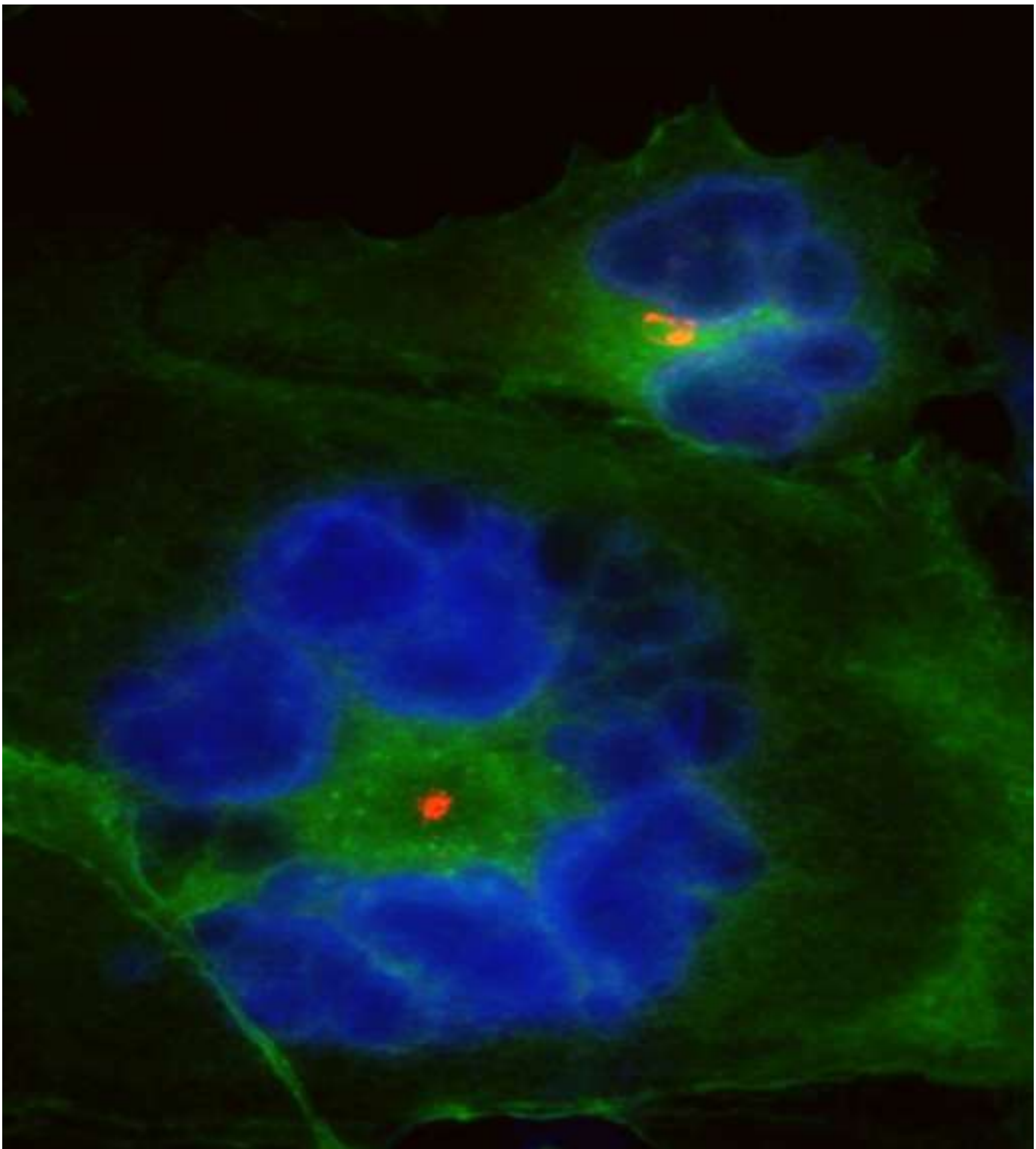

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LIPIDS

LI-P01
MICROSOMAL TRIACYLGLYCEROL TRANSFER PROTEIN (MTP) INHIBITION, BY HYPOCHOLESTEROLEMIC DRUG LOMITAPIDE, FAVORS TUMOR GROWTH

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Microsomal triacylglycerol transfer protein (MTP) locates in the lumen of the endoplasmic reticulum and participates in the secretion of lipids from the liver as very-low-density lipoproteins. There is evidence that MTP might be involved in other cellular processes, including the pathogenesis of different diseases; however, no studies were performed yet to evaluate whether MTP plays a role in cancer. The MTP inhibitor lomitapide binds directly to MTP, thereby inhibiting the synthesis of triglyceride-rich VLDL in the liver. Therefore, the objective of this work was to study the effect of MTP inhibition on tumor growth. Adult male Balb/c nude mice were subjected to a xenograft model where Huh7 cells (5 x 10^6 per mouse) were injected subcutaneously into the right flank of mice. Four days post-cell inoculation, mice were randomly divided into two groups (8 mice/group). One group (Control) received the vehicle (methylcellulose, gastric probe), and the other group received 5 mg/kg bw/day lomitapide (gastric probe) for 15 days. Tumors were monitored using a caliper, and volumes were estimated based on the formula "1/2 x length x width x height". At the end of the treatment, mice were sacrificed, and tumors were excised and weighed. After treatment, lomitapide-treated mice showed higher tumor volume and weight (2-fold) than control mice. Plasma levels of triacylglycerol and cholesterol were decreased (−30%, and −40%, respectively) in lomitapide-treated mice compared to control mice. Tumor histology analysis showed no differences between groups on tissue architecture and fibrosis; however, lomitapide-treated mice presented with an accumulation of cytosolic lipid droplets. Then, we evaluated proliferation by immunoblotting in total tumor homogenates. We found lomitapide-treated mice presented with increased protein expression of proliferation cell nuclear antigen (PCNA) (+58%) compared to control mice. In line, positive Ki-67-stained nuclei were increased in tumor sections from lomitapide-treated mice. In conclusion, these studies represent the first steps in the evaluation of the role of MTP in cancer development and demonstrate that MTP may be participating in tumor growth.

LI-P02
DOCOSAHEXAENOIC ACID EXERTS ANTIPROLIFERATIVE ACTIVITY ON PANCREATIC CELLS BY SHH AND IL-6 DOWNREGULATION

Garay MI[12], Barotto NN[2], Quiroga PL[2], Silva RA[2], Pasqualini ME[12].
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Pancreatic cancer (PC) remains one of the deadliest malignancies worldwide. PC is characterized by activation of the Sonic Hedgehog (SHH) signaling pathway as well as by an increment on IL-6 levels, a pro-inflammatory cytokine. Tumor cells can produce the SHH ligand that functions either in an autocrine or paracrine manner to promote tumorigenesis and survival of the tumors. Studies on many carcinomas demonstrated a ligand-dependent activation of Hedgehog (HH) signaling. Recent evidence has identified the importance of IL-6 in the regulation of SHH secretion in the tumor microenvironment. IL-6 is released in the tumor microenvironment, and its activation results in SHH expression, which, in turn, promotes the expansion of progenitor populations and leads to the re-growth of tumors. This evidence strongly supports the notion that IL-6 may facilitate the production and distribution of SHH to metastatic sites. On the other hand, growing evidence implicates fatty acid-induced signals as contributing to pancreatic carcinogenesis. Dietary ω3 polyunsaturated fatty acids (PUFAs) may have a protective role whereas ω6 PUFAs are associated with greater incidence and growth of pancreatic cancer. Nevertheless, neither the mechanism by which ω3 PUFAs induce suppression of pancreatic tumorigenesis or their effects on the SHH/IL-6 pathway have not been elucidated clearly. In the present work, we studied the effects of two omega-3 PUFAs, the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA) and the omega-6 fatty acid, the arachidonic acid (AA) on SHH/IL-6 expression in a human pancreatic cancer cell line (PANC-1) in order to find new chemotherapeutical approaches. We evaluated gene expression by qRT-PCR, protein production by immunofluorescence and ELISA, cell viability by fluorometry using Resazurin, migration by wound-healing, lipid cell profile by gas chromatography (GC), and eicosanoids generation by high-pressure liquid chromatography (HPLC), respectively. Our results showed that SHH and IL-6 expression were significantly downregulated by DHA in correlation with a decrease in cell viability and migration ($p < 0.05$). Furthermore, DHA induced a significant reduction in proliferative eicosanoids release: 12(S) HETE and 13(S) HODE ($p < 0.05$). Changes in membrane lipids induced by ω3 PUFAs may affect eicosanoid release, modulating cell signaling pathways of IL-6 and SHH. In this sense, we found that DHA downregulated both SHH and IL-6 expression, probably due to the decrease in pro-tumorigenic 12(S) HETE and 13(S) HODE eicosanoids leading to a diminution of pancreatic cell viability and proliferation.

LI-P03
PHOSPHOLIPASES A2: DISTINCTIVE ROLES IN THE REGULATION OF α-SYNUCLEIN BIOLOGY AND NEURONAL REDOX RESPONSE

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Iron (Fe) accumulation and α-synuclein (α-syn) overexpression are hallmarks of several neurodegenerative disorders. We have previously reported that Fe-induced oxidative stress activates fatty acid release catalyzed by different phospholipase A2 (PLA2) isoforms in the nervous system. In

this work, our aim was to study the involvement of PLA2s in the regulation of α-syn biology and the neuronal redox response to Fe overload. We also investigated the role of glia-secreted factors in the neuronal outcome. For this purpose, we exposed human neuroblastoma cells (IMR-32) to different ferric ammonium citrate concentrations (300–1000 μM) or vehicle for different incubation times (24–72 h). Using these experimental conditions, redox status, α-syn expression and phosphorylation, and the participation of calcium-independent and calcium-dependent PLA2 isoforms (iPLA2 and cPLA2, respectively) in the regulation of these events were studied. IMR-32 neurons exposed to Fe overload showed increased expression levels of iPLA2, concomitantly with an increase in lipid peroxides and reactive oxygen species. The pharmacological blockage of iPLA2 activity increased, even more, the levels of lipid peroxides and the content of reactive oxygen species. On the contrary, the inhibition of cPLA2 showed the opposite effect by promoting a decrease in oxidative stress markers associated with increased neuronal viability. Fe-challenged neurons also displayed increased α-syn expression and phosphorylation. The phosphorylation of α-syn was blocked by the inhibition of iPLA2 activity. To study the role of glia in the neuronal response to Fe, C6 astroglioma cells were challenged with ferric ammonium citrate or vehicle, and the astrocyte-derived media were added to neuronal cultures. Astrocytes exposed to Fe showed an increase in the glial marker S100B and lipid peroxidation levels, thus indicating reactivity to oxidative stress. Neurons incubated with the mentioned astrocyte-derived media displayed lower levels of oxidative injury than neurons only exposed to Fe. Astrocytes were positive for the rate-limiting step enzyme for glutathione biosynthesis. Altogether, our results show specific roles for the different PLA2 isoforms in the neuronal response to Fe-induced injury: whereas iPLA2 showed to be neuroprotective and also to be involved in the regulation of α-syn phosphorylation, cPLA2 appeared to act as a damage promoter. To ascertain the nature of the effect exerted by astrocytes on the neuronal response to oxidative stress, we are currently studying glutathione synthesis and how the isoform-specific PLA2-inhibition could be involved. *Sponsored by FONCyT, CONICET, UNS.*

LI-P04
SUPPLEMENTATION OF *EX VIVO* MOUSE TESTES EXPLANTS WITH PUFA-RICH LIPIDS STIMULATES SPERMATOGENESIS

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Using a gas-liquid interphase culture system from neonatal mouse testes, we previously observed in *ex vivo* explants a relationship between the progression of spermatogenesis at both cytological and histological levels and the gene expression of some of the enzymes involved in lipid metabolism. Here, we examined by RT-qPCR the expression of two PUFA elongases (*Elovl2* and *Elovl4*), Δ6-desaturase (*Fads2*), fatty acid 2-hydroxylase (*Fa2h*), two fatty-acid-binding proteins (*Fabp3* and *Fabp9*) and a diacylglycerol acyltransferase (*Dgat2).* Testis explants from 6 days old mice cultured for 22 days evidenced progress in spermatogenesis beyond the meiotic phase in some of the tubules. Although delayed *in vitro* in comparison with the *in vivo* development, in both cases the appearance of haploid germ cells occurred concomitantly with an increase in the expression of *Fabp9*, *Dgat2*, and *Fa2h*. Interestingly, the genes involved in PUFA synthesis (*Elovl2*, *Elovl4*, *Fads2*) and transport (*Fabp3*) were up-regulated in the testicular explants in comparison with the *in vivo* situation. This suggested, as a possible cause, partial insufficiency in the culture system of the C20-C22 PUFA required as substrates by these biosynthetic enzymes. This proved to be the case, as this medium contained low proportions (less than 4%) of these fatty acids. Supplementation of *ex vivo* explants with a PUFA-rich total lipid extract (TL) from adult mouse testis allowed progression into meiosis at the times in culture examined. Moreover, after 22 days in culture, the TL-supplemented explants contained more tubules with spermatogenic cells that had succeeded to reach the spermatid stage. Thus, in addition to growth factors and hormones, influences that promote the biosynthesis of PUFA-containing lipids are among the factors required to optimize spermatogenesis in *ex vivo* tissue explants. *Supported by FONCyT [PICT2017-2535] and PGI-UNS [24/B272] to GMO and by the MCIyU, Spain [BFU2017–87095-R] to JdM.*

LI-P05
IMPLICATION OF SPHINGOLIPIDS IN EPITHELIAL–MESENCHYMAL TRANSITION PROCESS IN RENAL COLLECTING DUCTS OF AGED RATS

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The epithelial–mesenchymal transition (EMT) is a process in which the cells lose their epithelial phenotype and acquire the characteristics of mesenchymal cells, which includes loss of cell–cell binding. Renal function declines progressively with age, and the EMT process has been suggested as a mechanism that drives renal fibrosis, with the consequent loss of tissue functions, which occurs mainly in old age. In previous works, we demonstrated that the inhibition of sphingomyelin synthase 1—the enzyme responsible for the synthesis of sphingomyelin (SM) at the Golgi Ap level—induces an EMT process in CD cells from the renal papilla of young, 70-day-old rats. We also demonstrated that the EMT occurs spontaneously in renal papillary CD cells of middle-aged rats (6–8 months), denoted by an impairment of cell–cell adhesion, a higher number of CD cells expressing the mesenchymal protein vimentin, and the *de novo* synthesis of α-smooth muscle actin (α-SMA), another mesenchymal biomarker. These results motivated us to study the possible implication of sphingolipids, and in particular SM, in the occurrence of EMT in renal papilla CD cells during aging. Taking into account that the cells in culture behave as in intact tissue, primary cultures of CD cells isolated from the renal papilla of young and middle-aged rats were performed. Since the occurrence of the EMT process was observed in 6-month-old rats, we performed a recovery experiment using the exogenous addition of 10 μM C12-SM to primary cultured CD cells from middle-aged rats. For this purpose, we simultaneously evaluated the intercellular adhesions by α- catenin immunostaining, and the expression of the mesenchymal biomarker α-SMA. After the addition of exogenous SM, the intercellular spaces between the CD cells disappeared, and α- catenin lined the lateral cell membranes, reflecting the presence of mature adherens junctions. Moreover, the percentage of CD cells that express α-SMA decreased (young vs middle-aged, $p = 0.0006$). We also analyzed the total SM content in CD cells isolated from young, middle-aged and aged-rats (15 months old) by thin-layer chromatography (TLC) and densitometry. Surprisingly, although we observed an EMT in CD cells from middle-aged rats, the quantitative results showed a decrease in SM content only in CD cells isolated from the renal papilla of aged rats (young vs aged-rats, $p = 0.0030$). Taking into account our previous and present results, we conclude that the epithelial-mesenchymal phenotypic conversion that spontaneously