VOL. 37
SUPPLEMENT, 2013

ISSN 0327 - 9545 (Print)
ISSN 1667 - 5746 (Electronic)
Printed in Argentina

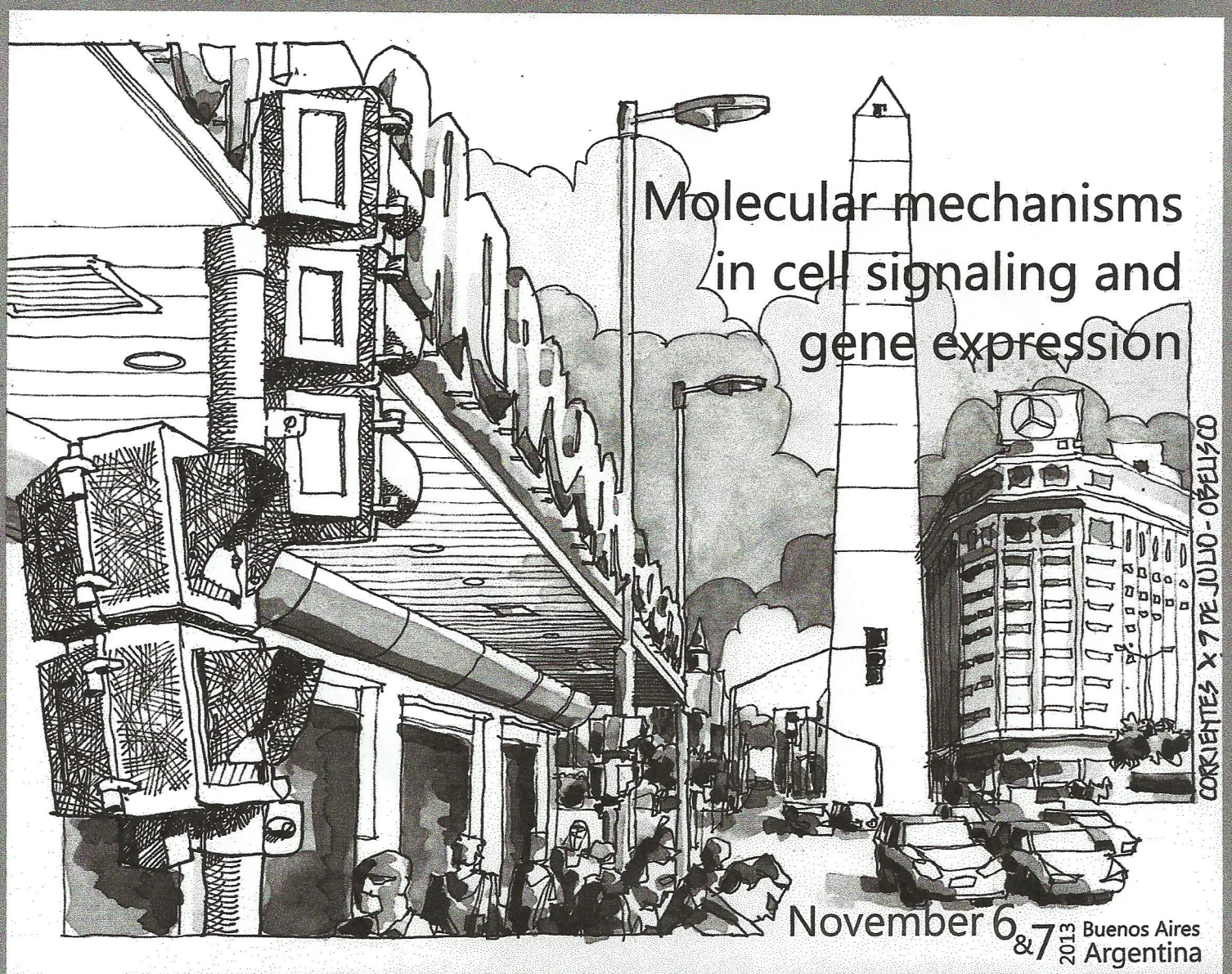
SUPPLEMENT

BIOCELL

SAIB

Argentine Society for Biochemistry and Molecular Biology Research

Sociedad Argentina de Investigaciones en Bioquímica y Biología Molecular



LI-P01.
INHIBITORY EFFECT OF n-3 POLYUNSATURATED FATTY ACIDS ON CANCER CELL GROWTH BY p53 ACTIVATION
Garay MI[1], Comba A[2], Vara Messler M[1], Silva RA[1], Quiroga PL[1], Pasqualini ME[1,2].
[1]Cat. Biol.Cel.Embr, Histol. FCM-UNC; [2]Inst. Invest. Cs. Salud (INICSA - UNC - CONICET).

Growing evidence show that n-3 and n-6 polyunsaturated fatty acids (PUFAs) have regulatory effects on carcinogenesis, however the mechanisms involved in this process remain unknown. In this report, we analyzed the *in vitro* and *in vivo* effects of n-3 and n-6 PUFAs on a syngeneic murine mammary adenocarcinoma cell line (LMM3). Tumor cells viability was measured by Resazurin and apoptosis by Hoechst. The PUFAs tumor cell membrane profile was analyzed by gas chromatography and PUFAs derivatives (eicosanoids) by HPLC. Our results showed that alpha linolenic acid (ALA, C 18:3 n-3) induced significantly higher pro-apoptotic effects than linoleic acid (LA, C18: 2, n-6). Moreover, ex-vivo studies showed that tumor cells from mice fed with ALA enriched diet diminished tumorigenesis by decreasing the tumor cell release of 12 (S)-HETE, a pro-carcinogenic eicosanoid and producing higher levels of pro-apoptotic 12(S)-HHT. In addition, the ALA enriched diet resulted on a higher tumor tissue expression of the pro-apoptotic tp53 tumor suppressor factor. These results provide evidence of the biological pro-apoptotic effects of n-3 PUFAs and particularly, the fatty acid ALA, as the regulator of the signaling pathway tp53. This provided a novel mechanism and a potential target on anticancer treatments.

LI-P02.
DOWNREGULATION OF GLI1-DEPENDENT SURVIVAL PATHWAY UNDERLIES ARACHIDONIC ACID ANTI-TUMORALACTIVITY
Comba A[1], Pasqualini ME[1], Vara Messler M[1], Silva RA[1], García Fernandez Barrena M[2], Fernandez Zapico ME[2], Eynard AR[1].
[1]INICSA-FCM-CONICET-UNC. [2]Mayo Clinic, Rochester, MN, USA.
E-mail: andreacomba@hotmail.com

Numerous studies have demonstrated a role for essential fatty acids (ePUFAs) during tumor development. However, the molecular mechanism underlying this phenomenon remains elusive. Here, we defined a novel molecular mechanism explaining the ePUFA arachidonic acid (AA) anti-tumoral activity. We used *in vivo* and *in vitro* assays to determine the effect of AA in primary tumor volume, lung micro-metastasis and apoptosis (TUNEL and Caspase 3/7 activation) as well as gene expression (qRT-PCR and WB) and transcriptional activity (Luciferase and ChIP assays). We observed a significant reduction in tumor volume and micro-metastasis incidence in AA-injected animals compared to the control group. Moreover, we demonstrated an increased apoptosis level in AA treated group *in vivo* and *in vitro*. Analysis of the mechanism showed that the AA treatment decreases the expression of the anti-apoptotic molecules Bcl-2 and Bfl-1/A1 by down-regulating their promoter activity. Moreover we found that the AA silencing of the oncogenic transcription factor GLI1 is the underling mechanism controlling Bcl-2 and Bfl-1/A1 expression. Finally, we demonstrated that AA-induced apoptosis can be rescued by overexpressing GLI1 in cancer cells. These results define a novel mechanism used by ePUFAs to inhibit tumor growth and suggest the use of AA for the development of new therapeutic approaches.

LI-P03.
MOLECULAR MECHANISM OF THE LIPIN1 ACTIVATION BY c-FOS
Cardozo Gizzi AM, Caputto BL.
Depto. de Química Biológica., CIQUIBIC (CONICET), Fac. de Cs. Químicas, UNC, Argentina. E-mail: acardozo@fcq.unc.edu.ar

The oncoprotein c-Fos activates phospholipid synthesis through a mechanism independent of its genomic AP-1 activity. To accomplish an overall activation of this synthesis, key metabolic steps are positively affected, including Phosphatidate Phosphohydrolase (PAP1). PAP1 catalyzes phosphatidic acid (PA) conversion to diacylglicerol. The mammalian enzymes responsible for PAP1 activity are the Lipin family, which are emerging as critical regulators of lipid metabolism. Lipins may govern the pathways by which phospholipids are synthesized and control the cellular content of signaling lipids.
Herein, we studied *in vitro* the activity of mammalian Lipin 1 (responsible of most PAP1 activity in mammals) purified to homogeneity. Lipin1 activity was measured with or without recombinant c-Fos in PA/Triton X-100 mixed micelles varying the assay conditions in order to clarify the activation mechanism. The kcat of the enzyme is doubled upon c-Fos addition, while the Km remains unaltered. Co-immunoprecipitation of the purified proteins demonstrated a physical interaction between c-Fos and Lipin 1. Using c-Fos mutants, we established the molecular determinants of the interaction. Results support our hypothesis that c-Fos physically associates with the phospholipid synthesis enzymes that it activates and reinforce the concept of a protein capable of increasing pivotal enzymatic activities *per se*.

LI-P04.
IMPLICATION OF SPHINGOLIPID METABOLISM IN RENAL EPITHELIAL CELL LINE DIFFERENTIATION
Santacreu BJ, Sterin de Speziale NB, Favale NO.
Fac. de Farmacia y Bioquímica, Universidad de Buenos Aires. IQUIFIB-CONICET. E-mail: bsantacreu@ffyb.uba.ar

Sphingosine 1-Phophate (S1P) is an important sphingolipid mediator in cell fate, synthetized by Sphingosine Kinase (SK). We studied the involvement of SK activity in the establishment of differentiated phenotype of MDCK cells induced by external hypertonic media. For this end, confluent MDCK cells were subjected to hypertonic medium with the concomitant addition or not (control) of D,L-threo-dihydrosphingosine (DHS) as an SK inhibitor. After 48 h of incubation, the cell phenotype was visualized by fluorescence microscopy, evaluating actin cytoskeleton and Adherens Junction (AJ) formation. DHS treatment induces β-catenin redistribution from plasma membrane to intracellular localization and actin cytoskeleton reorganization, resulting in disassembling of AJ. SK inhibition also induces an increase in *de novo* sphingolipid synthesis with Ceramide (Cer) accumulation. In order to evaluate whether AJ disassembly is due to Cer accumulation, Myriocin (Myr), an inhibitor of the novo synthesis, was used. Myr treatment recovers MDCK phenotype, suggesting that the disassembly of AJ due to inhibition of SK activity is an indirect effect produced by Cer accumulation.