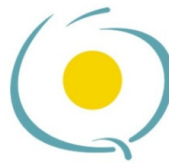
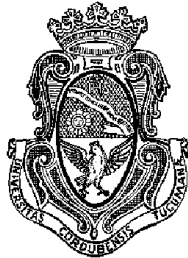


The background of the slide is a grayscale fluorescence microscopy image of cells. The cells are stained with three different dyes: green, blue, and red. The green staining highlights certain organelles or proteins, the blue staining (likely DAPI) highlights the nuclei, and the red staining highlights another set of organelles or proteins. The overall image is semi-transparent, allowing the text to be clearly visible.

SISTAM 2010

**The First South American Spring
Symposium in Signal Transduction and Molecular Medicine**
Córdoba, Argentina. October 24 – 28, 2010

www.sistam2010.com.ar



FACULTAD DE CIENCIAS QUÍMICAS



Genentech



Organizing Committee

J. Silvio Gutkind - NIDCR, NIH, Bethesda, MD, USA

Roger Davis - Univ. of Massachusetts, Worcester, MA, USA

Lucio Castilla - Univ. of Massachusetts, Worcester, MA, USA

Adalí Pecci - Universidad de Buenos Aires, Argentina

Omar A. Coso - Universidad de Buenos Aires, Argentina

Scientific Committee

José Maria Rojas - Instituto Carlos III, Madrid. Spain

Jose Badano - Instituto Pasteur de Montevideo, Uruguay

José Luis Bocco - Universidad Nacional de Córdoba, Argentina

Carlos Davio - Universidad de Buenos Aires, Argentina

Juan Olate - Universidad de Concepción, Chile

Claudia Tomes - Universidad de Cuyo, Argentina

José Vázquez – Prado – CINVESTAV, México

Meeting Manager: Silvina Ceriani

SISTAM Objectives

- To discuss recent advances in signal transduction research and the emerging opportunities to exploit this knowledge for the development of novel mechanism-based therapeutic options for different human diseases
- To provide an opportunity for local fellows to have first hand comments and input from top scientists in their research field
- To promote the establishment of long-term international collaborative efforts between local and visiting scientists in this exciting area
- To stimulate open scientific interactions among all attendees, as well as exploring research training options, including the recruitment of pre- and post-doctoral trainees

Speakers

XOSE BUSTELO - Cent. Inv. Cancer, Salamanca, SPAIN

LUCIO CASTILLA - Univ. of Mass., Worcester, MA, USA

JOHN CIDLOWSKI - NIEHS, NIH, NC, USA

MELANIE COBB - Univ. of Texas, SWMC., TX, USA

PIERO CRESPO - Univ. de Cantabria, SPAIN

ANA CUENDA - Univ. de Extremadura, Cáceres, SPAIN

ROGER DAVIS - Univ. of Mass., Worcester, MA, USA

CHANNING DER - UNC, Chapel Hill, NC, USA

JOZEF DULAK - Univ. Jagellonian, POLAND

CELINE GALES - Toulouse, FRANCE

MATHIAS GAESTEL - Hannover Medical School, GERMANY

BERND GRONER - Georg Speyer Haus Inst., GERMANY

J. SILVIO GUTKIND - NIDCR, NIH, Bethesda, MD, USA

NANCY HYNES - F.M. Inst. for Biomed. Res., SWITZERLAND

ANA ARANDA IRIARTE - Inst. de Inv. Med., Madrid, SPAIN

MARCELO KAZANIETZ - Univ. of Penn., PA, USA

ENRIQUE MESRI - Univ. of MIAMI, FL, USA

ANGEL NEBREDA - CNIO, Madrid, SPAIN

SARAH SPIEGEL - VCU, Richmond, VA, USA

HEIDI TISSENBAUM - Univ. of Mass., Worcester, MA, USA

YOSEF YARDEN - Weizmann Institute, ISRAEL

PABLO VISCONTI - Univ. of Mass., Amherst, MA, USA

Program

Two 60-minute Plenary Lectures

Three Poster Sessions

Eleven Oral Sessions with

Twenty Speaker presentations (40-minute talks) and

Eighteen Short Talks (15-minute presentations selected from the abstracts submitted for poster presentations by the Scientific Committee).

Sunday, October 24th

3:00 PM Registration opens at the main Lobby.

7:45 PM - 8:00 PM: Welcome speech

8:00 PM – 9:00 PM: Opening Lecture:

J. Silvio Gutkind, *National Institutes of Health, Bethesda MD, USA.*

***G PROTEIN-REGULATED SIGNALING NETWORKS IN CANCER PROGRESSION
AND METASTASIS:
A SYNTHETIC BIOLOGY APPROACH***

9:00 PM: Welcome Reception.

Monday, October 25th

7:00 AM - 8:30 AM Breakfast at the “Anexo Hotel FATAGA”.

8:40 AM – 10:50 AM Session # 1: all sessions will be held at the SUM Room
(a 5 minute interval is allocated between talks to allow for questions from the audience.)

8:40 AM – 9:20 AM: Lucio Castilla Univ. of Massachusetts Med. School, USA.

ONCOGENIC ROLE OF microRNA 17-92 CLUSTER IN ACUTE MYELOID LEUKEMIA

9:25 AM – 10:05 AM: Celine Gales INSERM, Toulouse, France.

NEW PARADIGM IN LIGAND-DIRECTED TRAFFICKING OF GPCRs: AT1 RECEPTOR EXAMPLE

10:10 AM – 10:25 AM: Juan Jose Bonfiglio. FCEN – University of Buenos Aires.

CRH SIGNALING IN THE CENTRAL NERVOUS SYSTEM: UNDERSTANDING MAPKs ACTIVATION

10:30 AM – 10:45 AM: Maria Julia Perez. FFyB – University of Buenos Aires. Argentina.

ACTIVATION OF FYN KINASE BY APOTRANSFERRIN IS ESSENTIAL FOR OLIGODENDROCYTE DIFFERENTIATION

10:50 AM – 11:20 AM Coffee Break.

11:20 AM -1:30 PM Session # 2:

11:20 AM – 12:00 PM: Jozef Dulak Jagiellonian University, Krakow, Poland.

OXIDATIVE SIGNALING, microRNA AND STEM CELLS

12:05 AM – 12:20 PM: Elba Vazquez FCEN – University of Buenos Aires. Argentina.

HO-1 AS A NEW PLAYER IN PROSTATE CANCER PROGRESSION

12:25 AM – 1:05 PM: Sarah Spiegel Virginia Commonwealth University, USA.

NOVEL INTRACELLULAR TARGETS OF SPHINGOSINE-1-PHOSPHATE

1:10 PM – 1:25 PM: Laila Suhaiman. Fac. Ccias. Med. UNCuyo-CCT, Mendoza. Argentina

EXOCYTOSIS IS TRIGGERED BY SPHINGOSINE 1-PHOSPHATE THROUGH THE ACTIVATION OF S1PR TYPE 1 AND 3

1:30 PM – 3:00 PM Lunch at the “Anexo Hotel FATAGA”.

3:00 PM – 4:50 PM Session # 3:

3:00 PM – 3:40 PM Nancy Hynes. F.M. Inst. for Biomed. Research, Switzerland.

TARGETING RECEPTOR TYROSINE KINASES IN BREAST CANCER

3:45 PM – 4:25 PM Yosef Yarden Weizmann Institute of Science, Rehovot, Israel.

ONCOGENIC TYROSINE KINASE NETWORKS: DEFECTIVE FEEDBACK REGULATION OF

SIGNAL TRANSDUCTION

4:30 PM – 4:45 PM Jimena Giudice. FCEN – University of Buenos Aires. Argentina.

SPATIO-TEMPORAL DYNAMICS OF ENDOCYTOSIS AND SIGNALING OF INSULIN RECEPTOR A AND B

4:50 PM – 5:20 PM Coffee Break

5:20 PM – 7:30 PM: Session # 4:

5:20 PM – 6:00 PM Angel Nebreda IRB-Barcelona, Spain.

SIGNAL INTEGRATION BY P38 MAPKS

6:05 PM – 6:20 PM Maria Sol Degese. FCEN – University of Buenos Aires. Argentina.

THE P38 MAPK PATHWAY PROMOTES cFOS mRNA DECAY

6:25 PM – 7:05 PM Mathias Gaestel Hannover Medical School, Germany.

MAPKAP KINASES MK2 AND MK3 IN INFLAMMATION AND BEYOND

7:10 PM – 7:25 PM María Victoria Goddio FCEN – University of Buenos Aires. Argentina.

THE RNA DESTABILIZING FACTOR AND TUMOR SUPPRESSOR GENE TTP IS INDUCED BY PROLACTIN IN MAMMARY CELLS

8:00 PM – 9:15 PM Dinner at the “Anexo Hotel FATAGA”.

9:15 PM – onwards Poster Session.

Tuesday, October 26th

7:00 AM - 8:30 AM Breakfast at the “Anexo Hotel FATAGA”.

8:40 AM – 10:50 AM Session # 5:

8:40 AM – 9:20 AM: Bernd Groner *Georg Speyer Haus Institute, Frankfurt, Germany.*

STAT3 AND STAT5: ONCOGENIC TRANSCRIPTION FACTORS AND TARGETS FOR TUMOR THERAPY

9:25 AM – 10:05 AM: Melanie Cobb *Univ. of Texas, Southwestern Med. Center, Dallas, USA.*

COMING FULL cERK1e ON LUNG CANCER

10:10 AM – 10:25 AM: Adriana De Siervi. *FCEN – University of Buenos Aires. Argentina*

CYCLIN T1: A NEW ONCOGENIC PROTEIN

10:30 AM – 10:45 AM: Guadalupe Lorenzatti, *FCQ – Universidad de Córdoba. Argentina*

SELECTIVE SIGNALING PATHWAYS PARTICIPATE IN THE REGULATION OF THE BIOLOGICAL ROLE OF ZEB1

10:50 AM – 11:20 AM Coffee Break.

11:20 AM - 1:30 PM Session # 6:

11:20 AM – 12:00 PM: John Cidlowski *NIEHS, NIH, Research Triangle Park, USA.*

THE GLUCOCORTICOID RECEPTOR: A SOUTH AMERICAN PERSPECTIVE

12:05 PM – 12:45 PM: Ana Aranda Iriarte *Inst. de Inv. Med., Madrid, Spain.*

THE THYROID HORMONE RECEPTORS AS REGULATORS OF CELL PROLIFERATION AND MALIGNANT TRANSFORMATION

12:50 PM – 1:05 PM: Luciana Rocha Viegas. *FCEN – University of Buenos Aires. Argentina*

ROLE OF UTX IN RAR-MEDIATED GENE REGULATION IN LEUKEMIA

1:10 PM – 1:25 PM: Virginia Novaro . *Inst. IBYME – Buenos Aires. Argentina.*

PI3K/AKT PATHWAY REGULATES HORMONE SENSITIVITY AND DIFFERENTIATION IN A MOUSE MAMMARY TUMOR MODEL

1:30 PM – 3:00 PM Lunch at the “Anexo Hotel FATAGA”.

3:00 PM – 7:00 PM Trekking and Networking.

7:00 PM – 8:00 PM Meet the Speakers

8:00 PM – 9:15 PM Dinner at the “Anexo Hotel FATAGA”.

9:15 PM – onwards Poster Session.

Wednesday, October 27th

7:00 AM - 8:30 AM Breakfast at the “Anexo Hotel FATAGA”.

8:40 AM – 10:50 AM Session # 7

8:40 AM – 9:20 AM: Channing Der Univ. of North Carolina, Chapel Hill, USA.

TARGETING THE RAL GUANINE NUCLEOTIDE EXCHANGE FACTOR (RALGEF)-RAL SMALL GTPASE EFFECTOR PATHWAY FOR THE DEVELOPMENT OF ANTI-RAS INHIBITORS FOR CANCER TREATMENT

9:25 AM – 10:05 AM: Piero Crespo Univ. de Cantabria, Santander, Spain.

SPATIAL REGULATION OF ERK1/2 MAP KINASES: NOVEL NUCLEAR AND CYTOPLASMIC EVENTS

10:10 AM – 10:25 AM: Giorgina Cardama . Univ. Nac. De Quilmes. Argentina.

STRATEGIES TO DEVELOP NOVEL ANTI-RHO GTPASE COMPOUNDS WITH ANTITUMORAL ACTIVITY

10:30 AM – 10:45 AM: Verónica Andreoli. FCQ – Universidad de Cordoba. Argentina

KLF6 TUMOR SUPPRESSOR ENGAGES C-JUN ONCOPROTEIN IN AN APOPTOTIC PATHWAY THROUGH JNK2 PHOSPHORYLATION

10:50 AM – 11:20 AM Coffee Break.

11:20 AM -1:30 PM Session # 8

11:20 AM – 12:00 PM: Pablo Visconti Univ. of Massachusetts, Amherst, USA.

PHOSPHORYLATION EVENTS DURING SPERM CAPACITATION

12:05 PM – 12:20 PM: Ernesto Podestá FMED – University of Buenos Aires. Argentina

TYROSINE PHOSPHATASE SHP2 REGULATES ARACHIDONIC ACID (AA) METABOLISM AND MITOCHONDRIA REARRANGEMENT

12:25 PM – 1:05 PM: Heidi Tissenbaum Univ. of Massachusetts Med. School, USA

USING *C. elegans* TO IDENTIFY NEW MODULATORS OF INSULIN/IGF-1 SIGNALING

1:10 PM – 1:25 PM Boccaccio, Graciela Fundacion Insituto Leloir. Argentina.

A KINASE AND PHOSPHATASE-WIDE RNAi SCREEN IDENTIFIES REGULATORS OF STRESS GRANULE DYNAMICS

1:30 PM – 3:00 PM Lunch at the “Anexo Hotel FATAGA”.

3:00 PM – 5:10 PM Session # 9

3:00 PM – 3:40 PM: Marcelo Kazanietz *Univ. of Pennsylvania, USA.*

DIACYLGLYCEROL AND RAC SIGNALING IN CANCER PROGRESSION

3:45 PM – 4:25 PM: Xose Bustelo *CIC, Salamanca, Spain.*

THE VAV ONCOPROTEIN FAMILY AS ANTI-CANCER TARGETS: POSITIVE AND NEGATIVE LIGHTS

4:30 PM – 4:45 PM Fiorella Galello *FCEN – University of Buenos Aires. Argentina*

IDENTIFICATION OF PROTEINS THAT INTERACT WITH PKA REGULATORY SUBUNIT FROM *Saccharomyces cerevisiae*

4:50 PM – 5:05 PM Maria Cecilia Carreras *FFyB – University of Buenos Aires. Argentina*

NITRIC OXIDE SYNTHASES TRAFFICKING INTO MITOCHONDRIA IN THE CONTROL OF CELL METABOLISM AND FATE

5:10 PM – 5:30 PM Coffee Break

5:30 PM – 6:30 PM: Plenary Lecture:

Roger Davis, *University of Massachusetts Medical School, Worcester MA, USA.*

METABOLIC STRESS REGULATION OF THE JNK SIGNAL TRANSDUCTION PATHWAY

6:45 PM 8:30 PM– Poster Session.

9:00 PM – onwards (Traditional Barbecue – ASADO)

Thursday, October 28th

7:00 AM - 9:00 AM Breakfast at the “Anexo Hotel FATAGA”.

9:00 AM – 12:00 PM Session # 10

9:00 AM – 9:40 AM: **Enrique A. Mesri** *Univ. of Miami, Miami, USA.*

A RAC1 MEDIATED OXIDATIVE STRESS LOOP IN PARACRINE VIRAL ONCOGENESIS OF KAPOSII'S SARCOMA IDENTIFIES NOVEL THERAPEUTIC TARGETS

9:45 AM – 10:15 AM: *Lic Guido Bonino, RAICES y Dr. Enrique A. Mesri, CAPICCyTE-RAICES y Consulado Argentino en Miami.*

ACTIVIDADES DEL PROGRAMA RAICES EN VINCULACION Y COOPERACION CIENTIFICA INTERNACIONAL. THE RAICES PROGRAM FOR INTERNATIONAL SCIENTIFIC COOPERATION WITH ARGENTINA

10:20 AM – 10:50 AM Coffee Break

10:50 AM – 11:30 AM: **Ana Cuenda** *Centro Nacional de Biotecnología/CSIC, Spain.*

ALTERNATIVE P38 γ AND P38 δ PATHWAYS IN THE IMMUNE RESPONSE

11: 35 AM – 11:50 AM: **Ruth Angélica Lezama Palacios,** *Escuela Nac. de Cs. Biologicas IPN-México.*

EVALUATION OF ANTI-APOPTOTIC PATHWAY IN BCR-ABL +/- ACUTE LYMPHOBLASTIC CELLS

11:50 AM – 12:00 PM: Closing Remarks:

Omar Coso, *University of Buenos Aires, Buenos Aires, Argentina.*

Farewell lunch and Meeting Adjourns – busses will transport participants to the airport.

ABSTRACTS

Speakers presentations

and

plenary lectures

J. Silvio Gutkind

N Oral and Pharyngeal Cancer Branch, NIDCR, National Institutes of Health,
Bethesda, MD 20892ational Institutes of Health, Bethesda MD, USA
gutkind@dir.nidcr.nih.gov

***G PROTEIN-REGULATED SIGNALING NETWORKS IN CANCER PROGRESSION
AND METASTASIS:
A SYNTHETIC BIOLOGY APPROACH***

With nearly one thousand members, the family of G protein-coupled receptors (GPCRs) constitutes the largest group of cell surface proteins involved in signal transduction. These receptors are activated by a diverse array of ligands, including peptide and non-peptide neurotransmitters, hormones, chemokines, growth factors, lipid metabolites, nucleotides, odorants and bitter and sweet tasting molecules, and even light photons. GPCRs participate in a wide variety of physiological functions, and they are also involved in a number of human diseases, as reflected by the fact that GPCRs are the target, directly or indirectly, of 50-60% of all current therapeutic agents. In this regard, the role of GPCRs in tumorigenesis is an area of active investigation. For example, the Kaposi's sarcoma (KS) associated herpesvirus (KSHV), the infectious cause of KS, the most common neoplasm arising in AIDS patients, expresses an oncogenic constitutively active GPCR, *vGPCR*, thus providing a direct link between G protein-linked receptors and viral-associated malignancies. Cancer oncogenome deep sequencing efforts have recently revealed multiple mutations in GPCRs and G protein α subunits in highly prevalent human cancers. Furthermore, GPCRs can contribute to tumor progression when persistently stimulated by tumor or stromal-released agonists. Indeed, malignant cells often hijack the normal physiological functions of GPCRs to survive, proliferate autonomously, evade the immune system, enhance their blood supply, invade their surrounding tissues, and disseminate to other organs. We have recently initiated a synthetic biology approach aimed at building, and hence understand, the GPCR-regulated signaling networks controlling cell proliferation, malignant transformation, and metastatic spread. The use of this experimental strategy to investigate how cancer cells co-opt chemokine networks and their G protein-initiated signaling circuitry to invade the surrounding tissues, increased vascular permeability, reach the vascular and lymphatic circulation, migrate and invade their target organs will be discussed. Ultimately, this knowledge may provide unique opportunities for cancer prevention and treatment.

Lucio Castilla

University of Massachusetts Medical School, Worcester, USA

lucio.castilla@umassmed.edu

ONCOGENIC ROLE OF *microRNA 17-92 CLUSTER IN ACUTE MYELOID LEUKEMIA*

Dmitri Madera and Lucio Castilla. Programs in Gene Function and Expression and Molecular Medicine, University of Massachusetts Medical School, Worcester MA, U.S.A.

The non-coding small RNAs microRNAs (miRs) are small RNAs that inhibit translation of proteins, regulating cellular processes associated with differentiation, proliferation and survival. The miR 17-92 cluster is a group of 6 miRs transcribed from a single mRNA. The miR-17 and -20a regulate myelo-monocytic differentiation by regulating transcription factor Runx1. Alternatively, miR-19 has been implicated in T-cell differentiation. When overexpressed, the miR cluster acts as an onco-miRs in solid tumors, and miR19 as the oncomiR responsible for T- and B-cell leukemias. We investigated the role of the miR17-92 cluster in acute myeloid leukemia (AML) expressing the fusion oncoprotein CBFbeta-SMMHC. The 17-92 miRs are upregulated in a fraction of human AML samples. The locus is a hot-spot of viral insertions in murine tumor cells, upregulating their expression. Transplantation assays of bone marrow cells expression of CBFbeta-SMMHC and the miR-cluster efficiently induced myeloid leukemia in mice. The leukemia cells show upregulation of miR-17, -20a, and -19, but not miR18 and -92. We show that expression of miR 17-92 cluster decreases expression of the phosphatase PTEN, increasing phospho-Akt. Inhibition of miR17/20 but not miR19, increases apoptosis and caspase-3 cleavage. Finally, inhibition of miR17/20 increases Bad protein levels. Together these studies show that miR17/20 family participate in AML development by increasing survival of leukemic cells, mediated by inhibition of PTEN. The implications of these findings on the complexity of miR regulation in normal hematopoiesis and leukemia development will be discussed.

Celine Gales
INSERM, Toulouse, France
Celine.Gales@inserm.fr

NEW PARADIGM IN LIGAND-DIRECTED TRAFFICKING OF GPCRS: AT1 RECEPTOR EXEMPLE

Aude Saulière¹, Hervé Paris¹, Colette Denis¹, Morgane Bellot¹, Jakob L. Hansen², Jean-Michel Sénard¹, Céline Galés¹. ¹INSERM U858, Team 8, Rangueil Institute of Molecular Medicine (I2MR), 1 avenue Jean Poulhès, BP 84225, 31432 Toulouse Cedex 4, France. ²Laboratory for Molecular Cardiology, Danish National Research Foundation Centre for Cardiac Arrhythmia, Department of Biomedical Sciences, Copenhagen University Hospital, Rigshospitalet, Juliane Mariesvej 20, section 9302, DK-2100, Copenhagen, Denmark.

Based on the concept of ligand-directed trafficking, one challenging task in pharmacology is now to optimize cellular assays to appreciate real texture of ligands. Thus, based on our previous work on G α i1 (Galés, 2006), we developed a BRET assay so measuring for the first time direct activation of all G protein isoforms from major G protein families (Gi, Gs, Gq/11, G12/13) in living cells. We then explored the activity of Sar1Ile4Ile8-AngII (SII) which has been described as a potent β -arrestin/ G protein independent biased agonist.

As already known, we found that AngII was able to activate all G protein isoforms with high efficacy on Gi and Gq families. Surprisingly, SII did also activate all G protein isoforms but to a lesser extent. When tested in G protein non overexpressing system, AngII and SII both promoted Ca²⁺ production thus validating activation of the Gq pathway. Interestingly, only SII directly inhibited cAMP production thus demonstrating direct cyclase inhibition through Gi proteins. However, AngII also promoted Gi coupling to AT1-R since PTX treatment revealed AngII-stimulating cAMP production. Thus, SII and AngII both appeared to activate Gi proteins but with different signaling outputs. Differences in G protein signaling for the two AT1 agonists were further reinforced by the observation that they both stabilized different conformations of AT1-R/Gq or Gi complexes.

Altogether, these results revealed that SII i/ coupled to G proteins, ii/ stabilized a new AT1-R conformation that signals differently from the natural and physiologic agonist AngII.

Jozef Dulak

*Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and
Biotechnology, Jagiellonian University, Krakow, Poland*
jdulak@mol.uj.edu.pl

OXIDATIVE SIGNALING, microRNA AND STEM CELLS

Protection of stem cells against oxidative stress is exerted by the high expression of antioxidant genes. However, besides cytoprotection, such genes can play much broader roles. One of the important enzymes is heme oxygenase-1 (HO-1) which degrades heme to carbon monoxide (CO), biliverdin, and ferrous iron. Beneficial activities of HO-1 have been recognized in cardiovascular system. Among them, our studies revealed the crucial role of HO-1 in modulation of VEGF and SDF-1 dependent blood vessel formation. HO-1 is required also for the functions of bone-marrow derived endothelial progenitor cells. Interestingly, our recent data indicate that HO-1, by regulation the expression of microRNAs, is involved in myoblast differentiation.

In this talk the pleiotropic functions of HO-1 will be presented with special emphasis on the significance of those mechanisms for stem cells and their potentials for regenerative medicine.

Sarah Spiegel

Virginia Commonwealth University, Richmond, USA

sspiegel@vcu.edu

NOVEL INTRACELLULAR TARGETS OF SPHINGOSINE-1-PHOSPHATE

Sarah Spiegel and Sheldon Milstien, Department of Biochemistry and Molecular Biology and the Massey Cancer Center, Virginia Commonwealth University School of Medicine, Richmond, Virginia 23298, USA.

Sphingosine-1-phosphate (S1P) is a potent sphingolipid mediator formed inside cells by phosphorylation of sphingosine catalyzed by two sphingosine kinase (SphK) isoenzymes, SphK1 and SphK2. The most well known actions of S1P are mediated by binding to a family of five specific G protein-coupled receptors (S1P₁₋₅). It has long been thought that S1P can function intracellularly independently of its cell surface receptors; however, until recently, direct intracellular targets of S1P were unknown. We have now identified several novel intracellular targets of S1P. We found that S1P formed in the nucleus by SphK2 specifically binds to the histone deacetylases, HDAC1 and HDAC2, and inhibits their enzymatic activity preventing the removal of acetyl groups from lysine residues within histone tails. SphK2 associates with HDAC1/2 in repressor complexes and is selectively enriched at the promoters of the genes encoding p21 and the transcriptional regulator c-Fos, where it enhances local histone H3 acetylation and transcription of these genes. Hence, S1P is the first identified endogenous small molecule regulator of HDAC1 and HDAC2, linking nuclear S1P to remodeling of chromatin and epigenetic regulation of gene expression (Hait et al, *Science* 325: 1254, 2009). More recently, we have shown that that SphK1 and production of S1P is necessary for Lys 63-linked polyubiquitination of RIP1, phosphorylation of IKK and I κ B α , and I κ B α degradation, leading to NF- κ B activation, independently of S1P receptors. S1P specifically binds to TNF receptor-associated factor 2 (TRAF2), a key component in NF- κ B signaling triggered by TNF- α at the N-terminal RING domain and stimulates its E3 ligase activity. S1P, but not dihydro-S1P, dramatically increased recombinant TRAF2-catalyzed Lys 63- but not Lys 48-linked polyubiquitination of RIP1 in vitro (Alvarez et al, *Nature* 465: 1084, 2010). This study reveals that TRAF2 is another novel intracellular target of S1P, and that S1P is the missing co-factor for TRAF2 E3 ubiquitin ligase activity, suggesting a new paradigm for regulation of Lys 63-linked polyubiquitination. These results also highlight the key role of SphK1 and its product S1P in TNF- α signaling and the canonical NF- κ B activation pathway important in inflammatory, anti-apoptotic, and immune processes. This work was supported by grants from the NIH R37GM043880 and R01CA61774, NIH RO1AI500941 and U19AI077435.

Nancy Hynes

*F.M. Inst. for Biomed. Research, Switzerland. Friedrich Miescher Institute for
Biomedical Research, Basel, Switzerland*
nancy.hynes@fmi.ch

TARGETING RECEPTOR TYROSINE KINASES IN BREAST CANCER

Breast cancer is a heterogeneous disease, from its histology to the array of expressed genes in individual tumors. Our molecular understanding of genetic alterations in breast cancer has increased significantly over the past years. Based on the identification of signaling pathways and proteins that are altered in breast tumors and are important for the clinical outcome of the patient, rational approaches for targeting breast cancer have become a reality. ErbB2, shown more than 20 years ago to be amplified and overexpressed in approximately 25% of breast tumors, has been successfully targeted using antibody-based approaches and tyrosine kinase inhibitors. We have been studying different receptor tyrosine kinases that are expressed in breast cancer in order to understand their role in the disease and to the potential for blocking them as an approach for cancer therapy. We have concentrated our recent efforts at uncovering new ErbB2 interacting proteins and testing their roles in the cancer phenotype. In this context we have identified Memo and Copine-III, both of which have roles in tumor cell migration, an essential part of the metastatic process. Ongoing work on the role of Memo in metastasis will be presented. Currently much effort is going into uncovering and targeting additional RTKs for breast cancer therapy. Based on the increasing evidence supporting the relevance of FGFRs in human breast cancer, we have explored the role of this receptor in various breast cancer models that display either autocrine FGFR activity or FGFR amplification. Treatment of these tumor cells in vitro and in vivo with small molecule FGFR kinase inhibitors blocks tumor cell proliferation and in vivo leads to a strong reduction of mammary tumor growth and lung metastasis. Ongoing work on the role of FGFRs as well as other receptor tyrosine kinases in breast cancer will be presented.

- Dey JH, Bianchi F, Voshol J, Bonenfant D, Oakeley EJ, Hynes NE (2010) Targeting Fibroblast Growth Factor Receptors blocks PI3K/AKT signaling, induces apoptosis and impairs mammary tumor outgrowth and metastasis. *Cancer Research* 70: 4151-4162.
- Heinrich C, Keller C, Boulay A, Vecchi M, Bianchi M, Sack R, Lienhard S, Duss S, Hofsteenge J, Hynes NE (2010) Copine-III interacts with ErbB2 and promotes tumor cell migration. *Oncogene* 29: 1598-1610.
- Meira M, Masson R, Stagljar I, Lienhard S, Maurer F, Boulay A, Hynes NE (2009) Memo is a novel cofilin interacting protein that influences PLCg1 and cofilin activities, and is essential for maintaining directionality during ErbB2 induced tumor cell migration. *J Cell Sci* 12: 787-797.

Yosef Yarden

Weizmann Institute of Science, Rehovot, Israel

Yosef.Yarden@weizmann.ac.il

**ONCOGENIC TYROSINE KINASE NETWORKS: DEFECTIVE FEEDBACK
REGULATION OF SIGNAL TRANSDUCTION**

Growth factors and their transmembrane receptors contribute to all steps of tumor progression, from the initial phase of clonal expansion (cell proliferation), through recruitment of blood vessels to growing tumors (angiogenesis), and, eventually to migration and colonization of distant organs (metastasis). Hence, the information relay system involved in growth factor signaling provides potential site for signal interception and tumor inhibition. A relevant example comprises the epidermal growth factor (EGF) and the respective receptor tyrosine kinases, namely ErbB-1/EGFR and HER2, which belong to a prototype signaling module that drives carcinoma development. The extended module includes two autonomous receptors, EGFR/ErbB-1 and ErbB-4, and two non-autonomous receptors, namely: a ligand-less oncogenic receptor, HER2/ErbB-2, and a kinase-dead receptor (ErbB-3). This signaling module is multiply involved in human cancer through autocrine loops involving co-expression of a receptor and one of the many EGF-like ligands, mutations and deletions within the *EGFR* gene, or amplification of either *HER2* or *EGFR*. Moreover, both EGFR and HER2 serve as targets for several cancer drugs, such as monoclonal antibodies (e.g., Cetuximab and Trastuzumab) and tyrosine kinase inhibitors (e.g., Erlotinib and Lapatinib).

To explain the remarkable oncogenic potential of HER2/ErbB-2, a ligand-less receptor that forms heterodimers with the other three ErbB proteins, we proposed a network configuration: through a layered organization of ligands, receptor dimers, downstream pathways and transcription factors, the ErbB network tunes and diversifies signal transduction, with HER2 operating as a signal amplifier. The network achieves robustness by adopting universal features common to engineered and natural systems: a modular architecture, a common core process, and a dense web of feedback control circuitry. My presentation will concentrate on system controls, which can be divided into two categories: the immediate loops are the domain of post-translational protein modifications, such as receptor phosphorylation, ubiquitylation and neddylation. One consequence of this phase comprises endocytosis of ligand-receptor complexes, a process evaded by several oncogenic mutants of EGFR. The late category of system control depends on newly transcribed messenger RNA and micro-RNA molecules. Through coordinated functions, inducible mRNAs and miRNAs terminate expression of the highly oncogenic immediate early genes, such as *c-FOS* and *c-JUN*. My lecture will highlight examples of tumor evasion from system control. In addition, I will focus on the inevitable fragility of robust signaling networks, as well as propose that system level understanding may help identify Achilles heels amenable for therapeutic intervention.

Angel Nebreda

IRB-Barcelona and ICREA, Barcelona, Spain

angel.nebreda@irbbarcelona.org

SIGNAL INTEGRATION BY p38 MAP KINASES

p38 α MAPK plays key roles in the cellular responses to stress and inflammation, but can also integrate signals that affect proliferation, differentiation, survival and migration in a cell context and cell type-specific manner. We have found that p38 α can modulate cancer cell-associated traits at multiple levels. Typically, normal cells use p38 α to suppress tumor initiation, by inducing either cell cycle arrest or apoptosis, whereas cancer cells can rewire this signaling pathway to facilitate proliferation, survival and invasion. We are also using genetically modified mice to investigate in vivo functions of p38 α and its roles in cancer development.

Mathias Gaestel

Hannover Medical School, Germany. Institute of Biochemistry, Hannover Medical School, Carl-Neuberg-Str. 1,30625 Hannover, Germany
gaestel.matthias@mh-hannover.de

MAPKAP KINASES MK2 AND MK3 IN INFLAMMATION AND BEYOND

Downstream of mitogen-activated protein kinases (MAPKs), three structurally related MAPK-activated protein kinases (MAPKAPKs or MKs) — MK2, MK3 and MK5 — signal to diverse cellular targets. Although there is no known common function for all three MKs, MK2 and MK3 are clearly involved in regulation of gene expression at the post-transcriptional level, control cytoskeletal architecture and cell-cycle progression, and are implicated in inflammation and cancer. MK2 and MK3 are phosphorylated and activated by p38MAPK-alpha/beta and, in turn phosphorylate various substrates involved in diverse cellular processes. In addition to forwarding of the p38-signal by MK2/3, protein complex formation between MK2/3 and p38 mutually stabilizes these enzymes and affects p38 signaling in general. Among the substrates of MK2/3, there are mRNA-AU-rich-element (ARE)-binding proteins, such as tris-tetraprolin (TTP), which regulate mRNA-stability and translation in a phosphorylation-dependent manner. Phosphorylation by MK2 stabilizes TTP and ARE-mRNAs by their exclusion from a default degradation pathway. MK2/3 also contribute to the de novo synthesis of TTP and of further immediate early genes by stimulating SRF-dependent transcription. Apart from this, MK2/3 bind to polycomb repressive complex and are involved chromatin remodeling necessary for stem cell renewal. Both p38 MAPK-alpha and MK2 are elements of TLR- and cytokine-signaling and are therefore preferential target molecules to treat chronic inflammation involved in asthma, rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, Alzheimer's disease, ischemic heart and brain diseases as well as cancer by orally available small molecules. Inhibitors against p38 MAPK have been tested in animal models and in the clinics, block acute and chronic inflammation efficiently, but show side effects such as liver toxicity and skin rash which might result from "on target"-effects. Thus, targets downstream to p38 MAPK-alpha, such as MK2, become more interesting for anti-inflammatory therapy.

Bernd Groner
Georg Speyer Haus Institute, Frankfurt, Germany
groner@em.uni-frankfurt.de

STAT3 AND STAT5: ONCOGENIC TRANSCRIPTION FACTORS AND TARGETS FOR TUMOR THERAPY

Vida Vafaizadeh, Petra Klemmt, Astrid Weiss, Corina Borghouts and Bernd Groner

The mammary epithelial ductal network initially consists of a single layer of luminal epithelial cells surrounded by myoepithelial cells in direct contact with the adjacent stroma. These cells proliferate and differentiate during pregnancy and form functional alveoli. Ductal luminal, alveolar luminal and myoepithelial cells originate from common, multipotent stem cell. Mammary gland development is regulated by systemically acting hormones, growth factors and cytokines. These signals activate transcription factors in mammary stem and progenitor cells, trigger differentiation and lineage commitment, influence the balance between proliferation, differentiation and apoptosis, but they also can contribute to cellular transformation and breast cancer. Estrogen is required for ductal elongation during puberty and progesterone and prolactin are mainly governing pregnancy and lactation. Prolactin mediates its functions through signal transducers and activators of transcription.

We have developed an efficient method to investigate the contributions of crucial signaling pathways to the functional potential of mammary stem cells (MaSCs). These cells are able to regenerate ductal structures upon transplantation into cleared fat pads. We studied the consequences of distinct genetic modifications of MaSCs on their repopulation and differentiation ability. The reconstitution of ductal trees was used as a stem cell selection procedure. MaSC, transduced with lentiviral gene transfer vectors prior to transplantation, resulted in transgenic ducts homogeneously expressing the virally encoded proteins. MaSC expressing different fluorescent proteins indicated that only a small number of individual MaSCs are required for glandular reconstitution. We also genetically modified MECs and defined functions of the Jak/Stat, the Hedgehog and the TGF- β pathways during mammary gland development and differentiation. Stat5-downregulation in MaSCs did not affect primary ductal outgrowth, but impaired side branching and the emergence of mature alveolar cells from luminal progenitors during pregnancy. Conversely, the expression of a constitutively active variant of Stat5 (cS5-F) caused epithelial hyperproliferation, thickening of the ducts and precocious, functional alveoli formation in virgin mice. Expression of cS5-F also prevented involution and caused the formation of estrogen and progesterone receptor positive adenocarcinomas. The tumors expressed activated Stat5 and Stat3 and contained a small fraction of CD44+ cells, possibly indicative of cancer stem cells. The Hedgehog pathway activation resulted in a loss of mammary epithelial differentiation potential and TGF- β inactivation in a more aggressive mammary tumor phenotype.

We also devised new therapeutic strategies based on the targeted inhibition of signaling components in breast cancer cells. The cellular delivery of peptides able to specifically interact with the functional activities of the oncoproteins Stat3 and survivin induced tumor cell death.

- "Stem cells of the breast and cancer therapy" B. Groner, V. Vafaizadeh; B. Brill and P. Klemmt *Women's Health Future Medicine* 6, 205-219 (2010)
- "Genetically modified mammary stem cells define distinct roles for Stat5 in mammary gland development and breast cancer" V. Vafaizadeh, P. Klemmt, C. Brendel, C. Döbele, Kara Britt, W.H. Chen, P. Bork, B. Korn, S. Desrivieres and B. Groner *Stem Cells* 28, 928-938 (2010)
- "The intracellular delivery of a recombinant peptide derived from the acidic domain of Pias3 inhibits Stat3 transactivation and induces tumor cell death" C. Borghouts, H. Tittmann, N. Delis, M. Kirchenbauer, B. Brill and B. Groner *Molecular Cancer Research* 8, 539-548 (2010)

Melanie Cobb

Univ. of Texas, Southwestern Med. Center, Dallas, USA

melanie.cobb@utsouthwestern.edu

COMING FULL *cERK1e* ON LUNG CANCER

We find that a subset of small cell lung cancers (SCLC) are dependent on the neuronal and pancreatic beta cell basic helix-loop-helix transcription factor NeuroD1 (aka BETA2) for proliferation and invasion in cell culture and xenograft assays. Among NeuroD1 targets in SCLC is the receptor tyrosine kinase TrkB (BDNF receptor). This receptor may be a useful drug target in some SCLC. Surprisingly, the MAPKs ERK1/2 are inactive in the majority of SCLC. In nonsmall cell lung cancer (NSCLC), ERK1/2 are often highly activated, consistent with frequent occurrence of mutations in Ras and other upstream genes in this form of the disease. I will discuss preliminary findings on the functions of ERK1/2 in NSCLC.

John Cidlowski
NIEHS, NIH, Research Triangle Park, N.C., USA
cidlows1@niehs.nih.gov

THE GLUCOCORTICOID RECEPTOR: A SOUTH AMERICAN PERSPECTIVE

John A. Cidlowski, Nick Z. Lu, Christine M. Jewell, Amy Beckley, Rongqin Ren, Javier Revollo, Erica Lannan, Danielle Duma, Kathy Gross, Shannon Whirledge, and Robert H. Oakley. Laboratory of Signal Transduction, NIEHS/NIH, Research Triangle Park, North Carolina, U.S.A.

Glucocorticoids are necessary for life after birth and regulate numerous homeostatic functions in man, including glucose homeostasis, protein catabolism, skeletal growth, respiratory function, inflammation, development, behavior and apoptosis. They are also one of the most prescribed classes of anti-inflammatory drugs in the world. Our understanding of how one hormone or drug regulates all of these diverse processes is limited, although most of these actions are thought to be mediated via the glucocorticoid receptor, which is a product of a single gene. However, recent studies in our laboratory have shown that multiple glucocorticoid receptor isoforms are produced from one gene via combinations of alternative mRNA splicing and alternative translation initiation. In addition these glucocorticoid receptor isoforms are subject to several post-translational modifications including ubiquitination, phosphorylation and sumoylation which also significantly modulate receptor function. In this lecture, I will discuss two new aspects of glucocorticoid receptor signaling that relate to sexually dimorphic responses to glucocorticoids and the role of transcriptional repressors in glucocorticoid regulation of metabolism. These discoveries reflect the effort of South American postdoctoral fellows currently in my laboratory.

Ana Aranda Iriarte

Instituto de Investigaciones Médicas, Madrid, Spain

aaranda@iib.uam.es

***THE THYROID HORMONE RECEPTORS AS REGULATORS OF CELL
PROLIFERATION AND MALIGNANT TRANSFORMATION***

The actions of the thyroid hormones are mediated by binding to nuclear receptors that regulate gene expression through the recruitment of transcriptional coactivators and corepressors. In addition to the well-known role of the thyroid hormone receptors (TRs) in growth, development and metabolism, there is increasing evidence that they can act as tumor suppressors. We have observed that TRs can block transformation and tumor formation by the *ras* oncogene. Mutant receptors that do not bind coactivators are able to display these actions, whereas receptors defective in corepressors binding are unable to antagonize the responses to the *ras*. Furthermore, expression of TR in hepatocarcinoma and breast cancer cells abolishes anchorage-independent growth and migration, blocks responses to growth factors and represses expression of pro-metastatic genes, reducing tumor growth and strongly inhibiting invasiveness and metastasis formation in nude mice. On the other hand, when cells are inoculated into hypothyroid animals, tumor growth is retarded but tumors are more invasive and metastatic growth is enhanced. Increased aggressiveness of skin tumors is found in TR K.O mice, demonstrating the role of the endogenous receptors as inhibitors of tumor progression. In contrast, TRs are required for normal skin proliferation. Reduced epidermal hyperplasia in response to proliferative stimuli, impaired hair growth and retarded wound healing are found in mice lacking TRs. These results appear to be a consequence of altered function of the epidermal stem cells in the K.O mice.

These results show the importance of TRs as regulators of normal proliferation and malignant transformation, and suggest that these receptors represent a potential therapeutic target in cancer.

Channing Der
Univ. of North Carolina, Chapel Hill, USA
cjder@med.unc.edu

**TARGETING THE Ral GUANINE NUCLEOTIDE EXCHANGE FACTOR (RalGEF)-Ral
SMALL GTPase EFFECTOR PATHWAY FOR THE DEVELOPMENT OF ANTI-Ras
INHIBITORS FOR CANCER TREATMENT**

Dominico Vigil, Nicole F. Neel, Timothy D. Martin, Tanya P. Zand, Jen Jen Yeh, David J. Reiner and Channing J. Der. University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center, Chapel Hill, NC 27514

Despite 28 years of intensive effort, currently no inhibitors of Ras have been developed successfully for cancer treatment. Currently, most efforts have focused on Ras effector signaling through the Raf-MEK-ERK MAPK cascade and the PI3K-AKT-mTOR pathway. However, our studies support the therapeutic importance of a lesser studied effector pathway, involving Ral guanine nucleotide exchange factor (RalGEF) activation of the RalA and RalB small GTPases. Our studies have focused on *KRAS* mutant pancreatic (PDAC) and colorectal (CRC) tumors. First, we determined that one RalGEF, Rgl2 is overexpressed in PDAC and required for Ral activation and growth in PDAC tumor cells. Second, we found distinct roles for the highly related RalA and RalB isoforms (82% identity) in PDAC growth, with RalA required for tumorigenic growth and RalB required for invasion and metastasis. Furthermore, we found that RalB but not RalA was essential for invadopodia formation in PDAC cells, through the RalBP1 effector pathway. Third, we observed striking cancer type differences in Ral function, where RalB functions as a tumor suppressor in CRC. Fourth, we found that RalA function was dependent on phosphorylation by Aurora-A whereas RalB function was dependent on phosphorylation by PKC α . These findings have prompted our evaluation of Aurora-A inhibitors for blocking RalA in PDAC. Finally, we utilized a genetic approach and determined that the RalGEF-Ral pathway antagonized the Raf-MEK-ERK cascade in *C. elegans* vulva development via altering cell fate. Taken together, our studies support highly context-dependent roles for the RalGEF-Ral pathway in promotion of Ras function.

Piero Crespo

Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC), Consejo Superior de Investigaciones Científicas (CSIC) - IDICAN - Universidad de Cantabria. Departamento de Biología Molecular, Facultad de Medicina. Santander, 39011. Cantabria. Spain

crespop@unican.es

SPATIAL REGULATION OF ERK1/2 MAP KINASES: NOVEL NUCLEAR AND CYTOPLASMIC EVENTS

Signals transmitted by ERK MAP Kinases are key orchestrators of essential cellular processes such as proliferation and survival. Upon stimulation, ERKs segregate into two distinct environments, the nucleus and the cytoplasm, where they regulate the functions of multiple substrates and are themselves subject to strict site-specific regulatory mechanisms, some of which shall be discussed herein. ERK1/2 MAP Kinases signals impact on cell cycle regulation as part of their role as orchestrators of cellular processes like proliferation. A-type lamins are major constituents of the nuclear lamina that also control the cell cycle machinery by largely unknown mechanisms. In an unprecedented functional liaison, insoluble lamin A serves as a mutually exclusive dock for ERK1/2 and the retinoblastoma protein. Immediately following their post-activation entrance in the nucleus, ERK1/2 dislodge Rb from its interaction with lamin A, thereby facilitating its rapid phosphorylation and, consequently, promoting E2F activation and cell cycle entry. Interestingly, this process does not occur with soluble lamin A, that regulates Rb functions independently of ERK1/2. These results indicate that space-related regulatory events occur even within the nucleus itself. In addition, several novel aspects of how scaffold-dimers interaction regulates ERK1/2 cytoplasmic events shall be discussed.

Pablo Visconti

Department of Veterinary and Animal Sciences, Univ. of Massachusetts, Amherst, USA
pvisconti@vasci.umass.edu

PHOSPHORYLATION EVENTS DURING SPERM CAPACITATION

Immediately after ejaculation, mammalian sperm are not able to fertilize. They will acquire fertilizing capacity after residing in the female tract for a period of time that varies depending on the species. The biochemical and physiological changes that occur to the sperm during this period and render these cells capable to fertilize are collectively known as capacitation. Although the molecular basis of this process is not completely understood, it is well established that activation/deactivation of proteins by phosphorylation plays an important role in the regulation of capacitation. At least in part, this process is mediated by protein kinase A (PKA) and depends on the activation of an atypical adenylyl cyclase (SACY) by HCO_3^- anions. This pathway is upstream of changes in protein tyrosine phosphorylation known to be associated with the capacitation process. It has been proposed that cSrc is the tyrosine kinase responsible for the observed capacitation-associated changes in tyrosine phosphorylation. Consistent with this hypothesis, cSrc inhibitors are able to block the increase in tyrosine phosphorylation. However, these inhibitors are also able to block phosphorylation by PKA without directly inhibiting PKA activity. Interestingly, this inhibition can be overcome with ser/thr phosphatase inhibitors such as okadaic acid at a concentration consistent with a role for PP2A in this process. These data suggest that two independent pathways are needed to induce changes in phosphorylation leading to capacitation. One of them relies on the activation of PKA, the other one depends on the inactivation of PP2A. Interestingly, activation of PKA or inactivation of PP2A, independently, is not sufficient to promote capacitation. Only after both pathways are induced, capacitation can proceed. Because cSrc is known to inhibit PP2A by phosphorylation in other systems, it is hypothesized that either cSrc or a related tyrosine kinase acts upstream PP2A and play a role in its inhibition during sperm capacitation.

Heidi Tissenbaum

University of Massachusetts Med. School, Worcester, USA

heidi.tissenbaum@umassmed.edu

USING C. elegans TO IDENTIFY NEW MODULATORS OF INSULIN/IGF-1 SIGNALING

Studies in mammals have revealed an intimate connection between insulin signaling, life span, and fat storage. Decreased levels of insulin signaling promote excess fat storage whereas obesity can result in insulin insensitivity. To dissect the molecular connections between insulin signaling, life span and fat storage, we use the nematode *C. elegans* as our model system. Worms are a system of choice for these studies since worms have a short reproducible life span and a well-conserved insulin/IGF-1 signaling pathway that modulates both lifespan and metabolism. Therefore, using *C. elegans*, we can dissect how modulating insulin levels results in changes in lifespan and fat storage on an organismal level. Thus far, in mammals as well as *C. elegans* the insulin signaling pathway has been shown to be regulated in large part through phosphorylation of its components. However, the phosphatases that regulate this pathway are limited. We performed an RNAi screen of Serine/Threonine phosphatases that modulate the IIS pathway. These phosphatases should function to counter-balance the effects of the kinases in the pathway. These phosphatases may regulate the insulin/IGF-1 signaling pathway or one of the multiple conserved pathways that couple the insulin/IGF-1 pathway. Over the long term, these studies should help to understand the complexities associated with diseases such as Type II diabetes. We will focus on the identification of two new phosphatases that modulate the insulin/IGF-1 pathway and regulate life span and metabolism.

Marcelo Kazanietz

University of Pennsylvania, USA. marcelo@spirit.gcrc.upenn.edu

DIACYLGLYCEROL AND Rac SIGNALING IN CANCER PROGRESSION

Cynthia Lopez-Haber, Hongbin Wang, Rachana Garg, Eva Wertheimer, Cinthia Roseblit, Mahlet Abera, Alvaro Gutierrez-Uzquiza, Maria Cecilia Caino, and Marcelo G. Kazanietz. University of Pennsylvania School of Medicine, Philadelphia, PA (USA)

Despite the extensive knowledge of the oncogenic signaling events that lead to the initiation and progression of cancer, a major goal is to identify novel signaling molecules that could be potential therapeutic targets. Protein kinase C (PKC) is a family of lipid-regulated serine-threonine kinases that has been broadly implicated in cancer progression and is the main direct effector of diacylglycerol (DAG), a lipid second messenger generated upon activation of tyrosine-kinases and GPCRs, and the phorbol ester tumor promoters. One of the complexities in DAG signaling is the multiplicity of targets for DAG/phorbol esters, including at least 7 PKC isozymes and Ras exchange factors. We established that DAG can also modulate in a dual manner the activity of the β 2-chimaerin, a specific GAP for the small GTPase Rac, via direct binding and indirectly through PKC-mediated phosphorylation. Chimaerin Rac-GAPs serve as negative modulators of Rac activation in response to the stimulation of growth factor receptors. There is a great need to dissect the specific roles of individual DAG effectors in cancer progression and cross-talks with other relevant tumorigenic pathways. The balance in PKC isozyme expression is markedly altered in human tumors. One of the most prominent changes is the vast up-regulation of PKC ϵ in epithelial cancers, such as breast, lung, and prostate cancer. We established that PKC ϵ drives survival responses in prostate and lung cancer cells via multiple mechanisms and opposes the responses of PKC δ , a pro-apoptotic/growth inhibitory kinase. PKC ϵ RNAi depletion markedly impaired the tumorigenic and metastatic activity of various cancer cell lines in nude mice. Moreover, PKC ϵ activation by growth factor receptors in lung and prostate cancer cells enhances motility via Rac. We generated a transgenic mouse model that overexpresses PKC ϵ specifically in the prostate epithelium under the control of a probasin promoter. These mice develop prostate hyperplasia and prostate intraepithelial neoplasia (PIN). Crossing PKC ϵ transgenic mice with Pten haplodeficient mice led to the development of invasive adenocarcinoma of the prostate. Immunohistochemistry analysis of these lesions revealed hyperactivation of the Akt, Erk, NF- κ B, and Stat3 pathways. A number of genes regulated specifically by PKC δ and PKC ϵ in prostate cancer have also been identified using microarray approaches. In another area of research, our laboratory focused on the elucidation of the mechanisms of Rac activation by ErbB receptors in breast cancer cells. Rac1 and its effectors are well-established mediators of breast tumorigenesis. However, little is known regarding the exchange factors (Rac-GEFs) that mediate ErbB receptor responses. We developed a "Rac-GEF" array to profile the relative expression of these exchange factors in breast cancer cell lines. This analysis identified the PIP3-G $\beta\gamma$ -dependent Rac-GEF P-Rex1 as highly expressed in breast cancer cell lines, particularly those of luminal origin. We found that P-Rex1 is highly overexpressed in human breast tumors, particularly those with high ErbB2 and ER expression, as well as in lymph node metastases. Using shRNA lentiviruses to specifically deplete P-Rex1 we established that this exchange factor is an essential mediator of Rac1 activation, motility, cell growth, and tumorigenesis driven by ErbB receptors in breast cancer cells. Notably, activation of P-Rex1 in breast cancer cells requires the convergence of inputs from ErbB receptors and a G $\beta\gamma$ /PI3K γ -dependent pathway downstream of the GPCR CXCR4. This transactivation mechanism is independent of the CXCR4 ligand SDF-1 α . In addition to the prognostic and therapeutic implications, these last findings reveal a novel ErbB effector pathway that is crucial for breast cancer progression.

Xosé Bustelo
CIC, Salamanca, Spain
xbustelo@usal.es

THE Vav ONCOPROTEIN FAMILY AS ANTI-CANCER TARGETS: POSITIVE AND NEGATIVE LIGHTS

Xosé R. Bustelo, Carmen Citterio, Mauricio Menacho. Centro de Investigación del Cáncer of Salamanca, CSIC–University of Salamanca, Campus Unamuno, 37007 Salamanca, Spain, EU

The Vav family is a group of signal transduction proteins with transforming potential that work downstream of tyrosine kinase–regulated pathways. This family contains single representatives in invertebrates and usually three members (Vav1, Vav2 and Vav3) in vertebrate species. The main known function of these proteins is to work as GDP/GTP exchange factors for Rho/Rac proteins, a family of Ras–related GTPases involved in a wide variety of intracellular functions such as cytoskeletal regulation, vesicle trafficking and cell proliferation. Under normal conditions, the enzyme activity of Vav proteins is stimulated by phosphorylation on tyrosine residues. This fine–tuned physiological regulation is lost when specific deletions or point mutations are created in the N–termini of Vav proteins, leading to the generation of highly active proteins whose biochemical activities are independent of upstream signals. Wild type Vav proteins can also become spuriously activated by overexpression, by tyrosine phosphorylation through autocrine loops, or through binding to virally encoded molecules. The alteration of this regulatory cycle may be of interest for human pathologies, since it has been shown that constitutively active Vav proteins are oncogenic and promote cancer–related processes such as cell cycle defects, aberrant cytoskeletal changes and metastasis. Genetic evidence derived from the use of knockout mice also supports the importance of Vav family function in the immune, nervous, and cardiovascular systems. Up to this moment, the implication of Vav proteins in tumorigenic processes has been based only on either *in vitro* culture experiments or on correlative associations derived from the detection of overexpressed family members in specific human tumors. However, it is hitherto unclear whether these proteins play a direct or just a collateral function in “real-life” tumorigenic states. To tackle this issue, we are using genetic approaches and Vav family knockout animals in our laboratory to establish the actual role of these proteins in different tumor types and, therefore, to set up their actual potential as drug targets. In this talk, we will provide an overview of these studies, giving information on the crucial role of Vav proteins on specific tumor types. In addition, we will provide evidence demonstrating that the three Vav family members share overlapping functions during tumorigenesis and, at the same time, that specific family members contribute preferentially to unique pro-tumorigenic signals. We will also show that the inhibition of these proteins leads to collateral effects in the cardiovascular system, leading to both hypertension and cardiovascular remodeling. However, those unwanted side effects can be easily controlled by the administration of anti-hypertensive drugs commonly used in the clinic, suggesting that those collateral effects will not represent a major concern when considering future anti-Vav family therapies in cancer.

This work was supported by grants to XRB from the NIH (5R01–CA73735), the Spanish Ministry of Science and Innovation (SAF2009-07172), the Red Temática de Investigación Cooperativa en Cáncer (RD06/0020/0001), the VII European Framework Program (FP7-HEALTH-2007-A-201862), the Castilla y León Autonomous Government (SA053A05 and GR97), and the Asociación Española contra el Cáncer.

Roger Davis

*Howard Hughes Medical Institute and Program in Molecular Medicine, University of
Massachusetts Medical School, Worcester, MA USA 01605, USA*

Roger.Davis@umassmed.edu

***METABOLIC STRESS REGULATION OF THE JNK SIGNAL TRANSDUCTION
PATHWAY***

The cJun NH2-terminal kinase 1 (JNK1) signaling pathway is implicated in the pathogenesis of diabetes. High fat diet-induced obesity causes activation of JNK1 in insulin target tissues and negative regulatory phosphorylation of IRS1. JNK1-deficient mice are resistant to the effects of feeding a high fat diet, including protection against insulin resistance and failure of obesity development. We have used tissue-specific JNK1-deficient mice to probe the mechanism of JNK1 regulation of insulin resistance and obesity. We show that JNK1 plays different roles in multiple tissues and that the phenotype of whole body JNK1-deficient mice reflects the interactions between these different JNK1-dependent processes. The molecular mechanisms of JNK1 function will be discussed.

Enrique A. Mesri
University of Miami, Miami, USA
emesri@med.miami.edu

A Rac1 MEDIATED OXIDATIVE STRESS LOOP IN PARACRINE VIRAL ONCOGENESIS OF KAPOSI'S SARCOMA IDENTIFIES NOVEL THERAPEUTIC TARGETS

Lucas E. Cavallin¹, Qi Ma^{1,2}, Pascal J. Goldschmidt-Clermont^{2*} and Enrique A. Mesri¹.¹Viral Oncology Program, Sylvester Comprehensive Cancer Center and Department of Microbiology & Immunology, ²Vascular Biology Institute, Department of Medicine, University of Miami Miller School of Medicine, Miami, FL 33136

Kaposi's sarcoma (KS), caused by the Kaposi's sarcoma-associated herpesvirus (KSHV), is a major AIDS-associated malignancy characterized by angiogenesis and proliferation of spindle cells. KSHV-infected KS lesions are composed of latently infected cells and a rather small minority of cells expressing lytic genes such as vGPCR, which are implicated in the development of KS angioproliferative phenotype via a paracrine mechanism (1,2). A key unsolved question is how this minor population of lytically-infected cells expressing vGPCR transduce an angio-proliferative phenotype to latently-infected cells, which do not express vGPCR and represent the majority of cells in the tumors. Here we describe a Rac1 and ROS driven mechanism of paracrine amplification of viral oncogenesis in the KSHV-induced tumor model mECK36 (3). It is triggered by vGPCR-induced PDGFB secretion, which in the presence of high expression of PDGFR-beta receptors and NADPH oxidase family members caused by latent KSHV infection, leads to STAT3-mediated transcriptional activation of c-Myc, VEGF and KSHV latent genes. This paracrine loop, driven by PDGF signaling and regulated by Rac1 and ROS, amplifies vGPCR oncogenesis by driving proliferation and angiogenicity in latently infected cells. The intervention of this molecular pathway by either the antioxidant N-acetylcysteine (NAC) or PDGF receptor tyrosine kinase inhibitors impair KSHV-induced tumorigenesis, angiogenesis and lymphangiogenesis, further validating this paracrine oncogenesis mechanisms and its components as anti-KS tumor targets.

- Bais C., Santomasso B, Coso O, Arvanitakis L, Geras Raaka E, Gutkind JS, Asch AS, Cesarman EC, Gershengorn MC and Mesri E.A. (1998) Kaposi's sarcoma associated herpesvirus (KSHV/ HHV-8) G protein-coupled receptor is a viral oncogene and angiogenesis activator. *Nature* 391: 86-89.
- 2. Bais C, Van Geelen A, Dias S, Silverstein RL, Rafii S and Mesri E.A. (2003). The KSHV G protein coupled receptor immortalizes human endothelial cells with upregulation of VEGF receptor-2/ KDR. *Cancer Cell*: 3:133-143.
- Mutlu A.M., Cavallin L, Vincent L., Chiozzini C., Eroles P., Duran E.M., Hooper A.T., Gao S-J, Dittmer D., Rafii S. and Mesri E.A. (2007) In vivo growth-restricted, reversible malignancy induced by Human herpesvirus-8/ KSHV: a cell and animal model of virally induced Kaposi's sarcoma. *Cancer Cell* 11:254-258.

Ana Cuenda

Centro Nacional de Biotecnología/CSIC, Madrid, Spain

acuenda@cnb.csic.es

ALTERNATIVE p38 γ AND p38 δ PATHWAYS IN THE IMMUNE RESPONSE

Based mainly on pharmacological experiments, it has been established that p38 α and/or p38 β are important regulators of normal immune and inflammatory response. However, the role of the related kinases, p38 γ and p38 δ in immunity has remained unclear. We found that deletion of both p38 γ and p38 δ impairs the innate immune response to the Toll-like receptor-4 ligand, lipopolysaccharide (LPS), by regulating the levels of upstream components of the ERK1/2 pathway in macrophages and dendritic cells (DC). In addition, the production of inflammatory cytokines was severely reduced in LPS-stimulated macrophages and DC derived from p38 γ/δ null mice. Furthermore, p38 γ/δ knockout mice showed prolonged survival times and significantly lower pro-inflammatory cytokine levels in sera upon intraperitoneal LPS challenge. Our results demonstrate an essential role of p38 γ and p38 δ in the innate immune response, identifying these kinases two possible new drug targets for the treatment of inflammatory diseases.

Short talks

P1.- CRH SIGNALING IN THE CENTRAL NERVOUS SYSTEM: UNDERSTANDING MAPKs ACTIVATION

Bonfiglio, Juan Jose¹; Senin, Sergio (1); Maccarrone, Giuseppina (2); Rewerts, Christiane (2); Refojo, Damián (2) ; Turck, Chris (2); Holsboer, Florian (2); Arzt, Eduardo (1); Silberstein, Susana (1) ¹Laboratorio de Fisiología y Biología Molecular, Departamento Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales (FCEN), Universidad de Buenos Aires e IFIBYNE-CONICET. ²Max-Planck Institute of Psychiatry, Munich.

jjbonfiglio@fbmc.fcen.uba.ar

Corticotropin-releasing hormone (CRH) is the key mediator of the neuroendocrine, autonomic and behavioral responses to stress. Our lab demonstrated that CRH, acting through its type 1 receptor (CRHR1), activates a MAPK signal transduction pathway downstream PKA in cultured pituitary corticotrophs and in vivo in specific areas of the mouse brain (hippocampal subfields and basolateral complex of the amygdala). We are exploring the signaling network that mediates MAPK activation by CRH in hippocampal cell lines using molecular and pharmacological tools combined with proteomics. We generated hippocampal HT22 clones stably expressing CRHR1. In these clones, CRH activates ERK1/2 through Rap1 and PKA (CRH 100nM; time: 1-60 min). We started to characterize the B-Raf interactome, the MAPKKK that belongs to the canonical MAPK signaling cascade in brain. Coimmunoprecipitated proteins with endogenous B-Raf were obtained from basal cell lysates of the HT22-clone2 and analyzed by LC-MALDI-MS/MS. This analysis allowed the identification of proteins of three classes: components of cytoskeleton, chaperones, and proteins involved in mRNA metabolism and translation. These results together contribute to the understanding of the molecular mechanisms involved in CRH signaling downstream CRHR1.

P2.-ACTIVATION OF FYN KINASE BY APOTRANSFERRIN IS ESSENTIAL FOR OLIGODENDROCYTE DIFFERENTIATION

Perez, María Julia¹; Pasquini, Juana María¹. ¹Dept of Biological Chemistry and IQUIFIB School of Pharmacy and Biochemistry. CONICET-UBA.

mjuliaperez@gmail.com

The Oligodendrocyte (OL) is the cell that produces the myelin membrane around axons in the CNS. Recently, we have observed that apotransferrin (aTf) addition to the culture medium accelerates the differentiation of OL. This effect involves an increase in the expression of the myelin basic protein (MBP). Fyn tyrosine kinase (Fyn) is a member of the Src family of proteins that has been shown to play an important role in myelination. We have recently showed that aTf added to OL primary cultures also activates Fyn at short times (i.e. 15min, 63 %). Studies were done in order to determine if Fyn is involved in the upregulation of the MBP promoted by aTf; the increased of MBP expression was abolished by the addition of PP2 (Fyn specific inhibitor) to the culture medium. In order to investigate the molecular mechanisms participating in this effect, we analyzed PI3K y MAPK signaling pathways since recent reports have demonstrated that active Akt increases myelination through the enhancement of OL maturation. Our results show that Akt is activated in the presence of aTf in the culture medium as well as ERK (i.e. 30 min; 273 % and 239 % respectively). These findings would suggest that aTf effects could be mediated by Fyn and Akt signaling.

P3.- HO-1 AS A NEW PLAYER IN PROSTATE CANCER PROGRESSION

Vazquez, Elba¹; Gueron, Geraldine¹; Ferrando, Mercedes¹; Elguero, Belén¹; Salles, Angeles¹; Meiss, Roberto²; Colombo, Lucas³; De Siervi, Adriana¹. ¹Department of Biological Chemistry, FCEyN, UBA-CONICET. ²National Academy of Medicine. ³Institute of Oncology A. H. Roffo - UBA.

elba@qb.fcen.uba.ar

Prostate cancer (PCa) is the second leading cause of cancer-associated death in men. The metastatic spread of PCa cells and the ability to survive when reaching the metastatic niche is affected by growth factors, angiogenic factors and hormones. Heme oxygenase-1 (HO-1) counteracts oxidative and inflammatory damage. Here we investigated its nuclear translocation in androgen sensitive and insensitive PCa cell lines. Our results showed that HO-1 overexpression induces its nuclear translocation. To understand HO-1 nuclear function, we analyzed its capacity to control androgen receptor (AR) targets. We found that HO-1 repressed the promoter activity of Prostate Specific Antigen (PSA), the best known AR downstream target and by ChIP we demonstrated that HO-1 associates to PSA promoter. HO-1 stable transfected PC3 cell lines (PC3HO-1) growing subcutaneously in athymic nude mice showed significant HO-1 nuclear staining. Using RT-qPCR-generated gene array we identified VEGFA as a novel downstream target of HO-1. An in vivo angiogenic assay also demonstrated that PC3HO-1 tumors presented less neovascularization than tumors derived from control cells. These results implicate for the first time HO-1 as a player in AR signaling and PCa progression.

P4.- EXOCYTOSIS IS TRIGGERED BY SPHINGOSINE 1-PHOSPHATE THROUGH THE ACTIVATION OF S1PR TYPE 1 AND 3

*Suhaiman, Laila²; De Blas, Gerardo A²; Obeid, Lina M¹; Darszon, Alberto³; Mayorga, Luis S²; Belmonte, Silvia A².*¹*Department of Medicine, Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, USA. ²Laboratorio Biología Celular y Molecular. Instituto de Histología y Embriología. Fac. Ccias. Med. UNCuyo. Mza. Arg. ³Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología.*

laila_suhaiman@hotmail.com

Regulated secretion is a central issue for the function of cells; for instance, mammalian sperm acrosomal exocytosis (AE) is essential for egg fertilization. Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid that regulates crucial physiological processes. S1P triggers AE in human sperm by a mechanism involving a Gi-coupled receptor, PLC and PKC activity. Real-time imaging showed an increase of cytosolic Ca²⁺ upon activation with S1P and pharmacological experiments indicate that the process requires extracellular Ca²⁺ influx and efflux from intracellular stores. Sphingosine kinase 1, the enzyme that catalyzes S1P synthesis, is not only present in human sperm but active and required for phorbol ester induced AE. Western blot and immunofluorescence demonstrated that S1P receptors 1 and 3 are present in human sperm cells. Specific agonists and antagonists of these receptors affected the AE suggesting that they are involved in this process. We present here the first piece of evidence indicating that S1P receptors are present in human spermatozoa and involved in the signal transduction cascade triggered by S1P leading to the acrosomal exocytosis.

P5.- Spatio-temporal dynamics of endocytosis and signaling of insulin receptor A and B

Giudice, Jimena^{1,2}; Coluccio Leskow, Federico¹; Arndt-Jovin, Donna³; Jovin, Thomas³; Jares-Erijman, Elizabeth². ¹Departamento de Química Biológica. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Argentina. ²CIHIDECAR. CONICET. Universidad de Buenos Aires. Argentina. ³Max Planck Institute for Biophysical Chemistry. Germany.

eli@qo.fcen.uba.ar

Insulin signaling is involved in glucose metabolism and in cellular growth. Impaired response to insulin is the hallmark of diabetes while upregulated insulin activity occurs in many cancers. Two splice variants of insulin receptor (IR) exist in mammals: IR-A lacking exon 11, involved in mitogenic signaling, and the full length IR-B responsible for the metabolic cascade. Although considerable biochemical data exist on insulin binding and signaling, little is known about the differential spatio temporal dynamics of IR isoforms and its possible effects on signaling. To gain insight into the dynamics of the activation and internalization of IR isoforms by microscopy we combined 2 techniques: streptavidin-quantum dots conjugated with biotinylated ligands; and visible fluorescent proteins. Using confocal and programmable array microscopy, we visualized endocytosis of both isoforms in living and fixed cells and in a cell-by-cell study we demonstrated a higher rate of endocytosis of IR-A compared to IR-B. These differences correlated with higher and sustained activation in response to insulin of IR-A, distinctive ERK activation profiles and different degree of stimulation of gene transcription suggesting a mechanism for the divergent regulation of gene expression in response to insulin.

P6.- THE p38 MAPK PATHWAY PROMOTES c-fos mRNA DECAY

Degese, María Sol¹; Tanos, Tamara¹; Naipauer, Julian¹; Rabinovich, Paula¹; Gutkind, Silvio²; Coso, Omar¹ IFIBYNE-CONICET, Laboratorio de Fisiología y Biología Molecular, DFBMC-FCEN- UBA, ARGENTINA. ²NIDCR, NIH, Bethesda, MD, USA.

soldgc@gmail.com

Cells respond to extracellular stimuli changing its metabolism, cytoskeletal structures and the protein repertoire. MAPK pathways constitute key regulatory elements for changes in the gene expression pattern. The expression levels of early responsive genes of the AP-1 family, as c-fos, peak shortly after cells are stimulated with growth factors and sharply decrease afterwards. Mechanisms that control rapid induction of c-fos promoter activity have been widely studied. On the other hand, much less is known regarding signaling pathways and c-fos mRNA decay. Proteins known as AUBPs bind to specific motifs (AREs) in the 3'-UTR region of mRNAs changing its stability. We observed that the stabilizing AUBP HuR binds to the c-fos ARE following a time course after growth factor stimulation, that peaks consistently with a maximum in the amount of c-fos mRNA present. We also observed that c-fos mRNA stability and the state of phosphorylation of HuR are dependent on p38 MAPK activity. In addition, the p38 pathway promotes HuR disassociation from the ARE. Our experiments support that while the ERK1/2 pathway is mainly responsible for c-fos promoter induction, activation of the p38 pathway inactivates the stabilizing effect of HuR and restores low levels of c-fos expression.

P7.- THE RNA DESTABILIZING FACTOR AND TUMOR SUPPRESSOR GENE TTP IS INDUCED BY PROLACTIN IN MAMMARY CELLS

Goddio, M. Victoria¹; Gattelli, Albana¹; Slomiansky, Victoria¹; Lacunza, Ezequiel²; Tocci, Johanna¹; Facchinetti, María Marta³; Curino, Alejandro³; Lamarre, Jonathan⁴; Abba, Martín C.²; Kordon, Edith C.¹. ¹IFIBYNE-CONICET, Dpto Qca Biológica, Facultad de Cs Exactas y Naturales, Universidad de Buenos Aires, CABA. ²CINIBA, Facultad de Ciencias Médicas, Universidad Nacional de La Plata. ³Laboratorio de Biología Básica del Cáncer, INIBIBB-CONICET, Centro Científico Tecnológico Bahía Blanca, Pcia. Buenos Aires, Argentina. ⁴Ontario Veterinarian College, University of Guelph, Ontario, Canada.

ekordon@qb.fcen.uba.ar

ARE-binding proteins (AUBPs) influence stability of specific mRNAs. Tristetraprolin (TTP), an AUBP whose expression is reduced in different cancer types, down-regulates cytokines and invasiveness-associated proteins. Breast cancer gene expression data sets showed TTP expression decrease associated to shorter survival. Then, we found that a significantly higher number of normal and hiperplastic breast samples display TTP positive immuno-staining compared to invasive carcinomas. Expression pattern in mouse mammary glands revealed strong TTP induction during lactation. Similarly, mammary HC11 cell differentiation in culture elicited expression of both, endogenous TTP and luciferase under the control of mouse TTP promoter sequences. Prolactin was required to trigger these effects and transfection with a Stat5a dominant-negative mutant abolished TTP induction. Prolactin also induced TTP in T47-D breast cancer cells, effect that was blocked by a JAK2 pharmacological inhibitor. Therefore, TTP expression is closely associated to mammary differentiation, being its transcription induced by prolactin-triggered STAT5 activation in both, human and mouse cells. We propose that this AUBP might play a relevant role during lactation and could exert hormone-driven tumor suppressor activities.

P8.- CYCLIN T1: A NEW ONCOGENIC PROTEIN.

De Siervi, Adriana¹; De Luca, Paola¹; Moiola, Cristian¹; Cotignola, Javier¹; Zalazar, Florencia¹; Gardner, Kevin²; Vazquez, Elba¹. ¹ Department of Biological Chemistry, FCEyN, UBA-CONICET. ² Laboratory of Receptor Biology and Gene Expression, National Cancer Institute, National Institutes of Health.

adesiervi@qb.fcen.uba.ar

Human Positive Transcription Elongation Factor b (PTEFb) is a protein kinase composed by CDK9 and Cyclin T that controls the elongation phase of RNA Pol II. This complex also affects the activation and differentiation program of lymphoid cells. In this study we found that several head and neck tumor cell lines overexpress PTEFb. We also established that Cyclin T1 is able to induce transformation in vitro, as we determined by foci and colony formation assays. *Nu/nu* mice s.c. injected with stable transfected Cyclin T1 cells (NIH 3T3 Cyclin T1) developed tumors faster than animals injected with control cells (NIH 3T3 B-gal). *In vitro*, NIH 3T3 Cyclin T1 cells show increased proliferation and CDK4-Rb phosphorylation. Even more, silencing E2F1 (shRNA E2F1) in NIH 3T3 cells resulted in a dramatic inhibition of Cyclin T1-induced foci. All these data demonstrate for the first time the Cyclin T1 oncogenic function and suggest a role for this protein in controlling cell cycle probably via Rb/E2F1 pathway.

P9.- SELECTIVE SIGNALING PATHWAYS PARTICIPATE IN THE REGULATION OF THE BIOLOGICAL ROLE OF ZEB1

Lorenzatti, Guadalupe¹; Cabanillas, Ana María¹. ¹ CIBICI-CONICET, Dpto. de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba (UNC), Argentina.

glorenzatti@fcq.unc.edu.ar

ZEB1 is highly involved in metastasis by repressing E-cadherin. We reported that hypophosphorylated (PO4) ZEB1 represses more strongly its target genes and that the PO4 sites lie in its C-term, ZD2. Previous data showed that IGF-1 regulates ZEB1. We aimed to identify key PO4 sites of ZEB1 and to study which IGF1 downstream pathways, PI3K or MEK/ERK, are involved in ZEB1 role. We generated 3 blocks of ZD2 mutants (M). EMSA & reporter assays were used to test binding and biological role of M. Control ZD2wt or the M and E-cadherin or ZEB1-luciferase promoters were transfected to CHO-K1 cells. ZD2-2 M did not change promoters activities respect to ZD2wt but, ZD2-1A, -1B, -3A and -3C repressed luciferase more than ZD2wt. Incubation w/MEK/ERK or PI3K inhibitors increased the repression induced on ZEB1 promoter by ZD2wt and ZD2-2 M, but not by the other M. No higher repression was induced by LY294002 on E-cadherin promoter. Treatment w/ a PKC activator lowered repression induced by ZD2wt but not by ZD2-1 and -3. These unresponsive M did not increase their binding to ZEB1 target genes after treatment w/ alk. phosphatase (CIP) in EMSA. Our results point that M ZD2-1A, B & -3A, C harbor key ZEB1 PO4 sites which are likely targets of MEK/ERK pathway for the repression of E-cadherin by ZEB1.

P10.- ROLE OF UTX IN RAR-MEDIATED GENE REGULATION IN LEUKEMIA

Rocha Viegas, Luciana^{1,2}; Villa, Raffaella²; Iriando, Ohiana²; Gutierrez, Arantxa²; Di Croce, Luciano². ¹DFBMC e IFIBYNE-CONICET, FCEN, UBA. ²Centro de Regulación Genómica (CRG), Barcelona, España.

chocha_76@yahoo.com

Human UTX, a member of the Jumonji C family of proteins, associates with mixed-lineage leukemia (MLL) 2/3 complexes, and during retinoic acid signaling events, the recruitment of the UTX complex to HOX genes results in H3K27 demethylation and a concomitant methylation of H3K4. Here we show that UTX interacts with the retinoic acid receptor (RAR) and is involved in the differentiation of leukemic U937 cells in response to RA. UTX occupies the promoters of key genes linked to differentiation and regulates their transcriptional output by modulating the recruitment of MLL 2/3 and ASC2 complexes. Moreover, overexpression of UTX in promyelocytic NB4 cells results in enhanced cellular differentiation upon RA treatment. Our results suggest a concerted mechanism for transcriptional activation during differentiation in which cycles of H3K4 methylation by MLL2/3 are linked with demethylation of H3K27 through UTX at RA-responsive genes.

P11.- PI3K/AKT PATHWAY REGULATES HORMONE SENSITIVITY AND DIFFERENTIATION IN A MOUSE MAMMARY TUMOR MODEL

Novaro, Virginia¹; Riggio, Marina¹; Polo, María Laura¹; Colman-Lerner, Alejandro²; Blaustein, Matías²; Giulianelli, Sebastián¹; Lanari, Claudia¹. ¹Laboratorio de Carcinogénesis Hormonal, IBYME-CONICET. ²Laboratorio de Fisiología y Biología Molecular LBFM, IFIBYME (FCEN-UBA).

vnovaro@gmail.com

Using a mouse mammary tumor model induced by medroxyprogesterone acetate (MPA) that transits through different stages of hormone dependence, we have previously reported that in the progression from hormone-dependent (HD) into a hormone-independent (HI) phenotype, the PI3K/AKT pathway becomes critical. The objective of this work was to explore if the activation of PI3K/AKT pathway in the HI tumor is responsible in regulating cellular proliferation and differentiation. We were able to generate mammary tumors in nude mice even in the absence of MPA by inoculating HD tumor cells expressing a constitutively active form of AKT1 (myristoylated AKT1). These tumors are highly differentiated and display a ductal phenotype with a cytokeratin 8 and laminin-1 positive pattern, two characteristics typical of HI tumors. Furthermore, we demonstrate that by expressing myristoylated AKT1 in a subpopulation of epithelial cells, neighboring epithelial cells respond with an increase in endogenous AKT1 levels. These results indicate that the activation of PI3K/AKT pathway is decisive in the generation of an autonomous tumor, and that paracrine signals derived from the tumor cells or its microenvironment might participate in the activation of the signaling pathway and ultimately in HI tumor growth.

P12.- STRATEGIES TO DEVELOP NOVEL ANTI Rho GTPase COMPOUNDS WITH ANTITUMORAL ACTIVITY

*Cardama, Georgina¹; Lorenzano Menna, Pablo¹; Alonso, Daniel¹; Gomez, Daniel¹.¹
Laboratorio de Oncología Molecular, Universidad Nacional de Quilmes.*

plmenna@unq.edu.ar

Rho GTPases belong to the Ras superfamily with Rac1 being one of the most studied members. These proteins regulate many essential cellular processes, including actin dynamics, gene transcription, cell-cycle. Aberrant signaling by Rho GTPases has been found to be involved in a wide range of diseases, including cancer. Thus, Rho proteins appear to be promising targets for the development of novel anticancer drugs. In our laboratory, we have developed different strategies for the discovery of novel anti Rho GTPase compounds. The first strategy was to design GTP analogs with potential affinity for the Rac GTP binding site. These analogs showed antitumoral activity in vitro and in vivo in a mouse mammary carcinoma model. The other strategy consisted in a docking-based virtual library screening having Rac1 as molecular target. We searched for specific Rac inhibitors able to block the Rac-Tiam interaction, preventing Tiam-mediated activation of Rac1. We have already tested a dozen lead-compounds in vitro and at least four of them showed antitumoral activity. One of these compounds is able to reduce Rac1 activation levels and showed to have a strong impact on several Rac1-mediated cellular processes such as migration, actin polymerization, apoptosis and cell cycle on a human glioblastoma.

P13.- KLF6 TUMOR SUPPRESSOR ENGAGES c-Jun ONCOPROTEIN IN AN APOPTOTIC PATHWAY THROUGH JNK2 PHOSPHORYLATION

Andreoli, Verónica¹; Petiti, Juan Pablo²; Trucco, Lucas Daniel¹; Torres, Alicia Inés²; Bocco, José Luis¹. ¹ Dpto. Bioquímica Clínica (CIBICI). ² Centro de Microscopía Electrónica, Fac. de Cs. Médicas, Universidad Nacional de Córdoba.

vandreoli@fcq.unc.edu.ar

Krüppel-like Factor 6 (KLF6) expression is responsive to external cues mediated by several stimuli though the underlying mechanisms and how they are integrated with cell biology are largely undeciphered. According to the stimulus detected by the cell, KLF6 contributes to reprogram the transcriptional response and/or interact functionally with other transcription factors like c-Jun to organize the adaptive cell response (e.g. proliferation rate or apoptosis) in a coordinated fashion. Interestingly, after DNA damage signaling KLF6 promotes apoptosis involving sustained phosphorylation of c-Jun by JNK2. KLF6 expression improves JNK2 activity leading to a transient increase of c-Jun phosphorylation in cells lacking JNK1 activity, correlating with KLF6 nuclear translocation. In this particular context of *jnk1*^{-/-} cells, expression of KLF6 was directly associated with increased levels of p53 transcript, induction of p73 promoter, activation of caspases-3/-7 and increased cellular labeling with Annexin-V. Additionally, experiments are consistent with the idea that KLF6 exploits the pro-apoptotic properties described for the hyper-phosphorylated form of the c-Jun oncoprotein which in turn is essential to promote the "switch" of p53 function from cell cycle arrest towards apoptosis.

P14.- Tyrosine phosphatase SHP2 regulates arachidonic acid (AA) metabolism and mitochondria rearrangement

Duarte, Alejandra¹; Poderoso, Cecilia¹; Cooke, Mariana¹; Orlando, Ulises¹; Cornejo Maciel, Fabiana¹; Soria, Gastón²; Gottifredi, Vanesa²; Podestá, Ernesto¹. ¹IIMHNO and Department of Biochemistry, School of Medicine, University of Buenos Aires. ²Fundación Instituto Leloir, Buenos Aires, Argentina.

ernestopodesta@yahoo.com.ar

A previous report from our laboratory showed a new mechanism that controls the level of intramitochondrial AA and its conversion to lipoxygenase products in steroidogenic and cancer cells. This mechanism involves the action of an acyl-CoA synthetase (ACSL4) a key enzyme in the regulation of the aggressive phenotype in cancer cells that is regulated via the activation of Tyrosine Phosphatases (PTPs). Using overexpression and suppression approaches, we demonstrate that SHP-2 is, at least, one of the PTPs that play an obligatory role in this regulation. We have also demonstrated that activation of the cAMP signal leads to a translocation of PKA and ERK to the mitochondria. However, there is no description of a mitochondrial target signal in PKA and ERK. We observed that hormone treatment of the cells results in a rearrangement of mitochondria and that SHP2 knock-down abolishes the movement of the mitochondria and the translocation of ERK. Moreover the rearrangement of the mitochondria produces the association of ACSL4 and the microsomal associated membrane with the mitochondria. Thus, it seems that SHP-2 may control the mitochondrial movement to allow for the rearrangement of crucial mitochondria proteins involved in the generation of a protein complex that regulate cellular function.

**P15.- A KINASE AND PHOSPHATASE-WIDE RNAi SCREEN IDENTIFIES
REGULATORS OF STRESS GRANULE DYNAMICS**

*Loschi, Mariela¹; Slomiansky, Victoria¹; Thomas, M. Gabriela¹; Vázquez, M. Soledad¹;
Boccaccio, Graciela L.¹. ¹ Instituto Leloir, IIBBA CONICET and FCEyN, UBA- Argentina.*

GBoccaccio@leloir.org.ar

Stress granules (SGs) are cytoplasmic foci that form transiently during acute stress. SGs contain repressed mRNAs and RNA-binding proteins involved in reprogramming mRNA translation and decay, and also sequester key modulators of cell survival and proliferation, all this helping to avoid apoptosis. SG assembly and disassembly is a multi-step process that depends on: i) translation inhibition (Thomas et al, 2005; 2009; 2010). ii) Retrograde transport mediated by dynein (Loschi et al. 2009). iii) Aggregation by specific protein domains. iv) Dispersion mediated by kinesin (Loschi et al., 2009). We performed a RNAi-based screen in *Drosophila* cells of the 468 genes encoding kinases and phosphatases present in the fly genome to identify signalling pathways regulators of SG dynamics. The KD of the eIF2alpha kinases CGN2 or PEK impaired SG assembly, whereas the KD or pharmacological inhibition of the cognate phosphatase PP1 α ; affects SG dissolution. KD of p38b or upstream kinases affect SG dissolution, whereas JNK KD has no effect, in agreement with the effect of p38 and JNK inhibitors in mammalian cells. This kinome-phosphatase screen opens new lines of research on SG dynamics. We thank the *Drosophila* RNAi screening center, Harvard Medical School, and ANPCyT, CONICET and UBA, Argentina, and FIRCA-NIH, USA, for funding.

P16.- IDENTIFICATION OF PROTEINS THAT INTERACT WITH PKA REGULATORY SUBUNIT FROM SACCHAROMYCES cerevisiae

Galello, Fiorella¹; Moreno, Silvia¹; Rossi, Silvia¹. ¹Laboratorio de Biología Molecular y Transducción de Señales Depto Química Biológica-FCEN-UBA.

fgalello@qb.fcen.uba.ar

Subcellular targeting through the association with adaptor and scaffolding proteins has emerged as a key mechanism by which cells maintain signaling specificity. Compartmentalization of cAMP-PKA pathway is maintained by the clustering of cAMP signaling enzymes in discrete units by the A kinase anchoring proteins (AKAPs) through their interaction with regulatory subunit (R). No anchoring proteins had been characterized up to now in *S. cerevisiae*. We have identified proteins that tether regulatory subunit (Bcy1) from yeast PKA using TAP-affinity purification and MALDI-TOF identification. The interactions were assessed by pull-down and peptide arrays. Although α -helices were predicted in all domains involved in the interaction with Bcy1, they do not present a perfect hydrophobic face. Similar residues to those that shape the binding pockets in mammalian AKAPs are present. The importance of these residues was assayed using peptide arrays. Positive charged residues present in the domain are determinant for the interaction. We demonstrate using BCY1 N-terminus mutants that the region 1-138 is necessary for the interaction with these proteins. The interaction in vivo was corroborated by pull-down and immunoprecipitation assays.

P17.- NITRIC OXIDE SYNTHASES TRAFFICKING INTO MITOCHONDRIA IN THE CONTROL OF CELL METABOLISM AND FATE

Carreras, Maria Cecilia. Laboratory of Oxygen Metabolism, Hospital de Clínicas, University of Buenos Aires. Department of Clinical Biochemistry, School of Pharmacy and Biochemistry. CONICET.

carreras@ffyb.uba.ar

We previously reported increased mitochondrial translocation and activation of neuronal nitric oxide synthase (nNOS) in experimental hypothyroidism and by activation of insulin-PI3K/Akt pathway. nNOS is imported and activated by a complex mechanism involving endogenous proteases, chaperones and kinases. Either in vivo or in vitro (transcription-translation import assays or cell transfection), we observed a reduction of the apparent molecular weight of the protein from 157 to 130 kDa during import. This finding results from Ca-dependent activation of calpain1 that cleaves the N-terminal domain losing the PDZ region, favoring mitochondrial nNOS import. Mitochondrial nNOS is phosphorylated in Ser1412 by P-Akt. Phosphorylation increases nNOS activity by a positive allosteric effect. On the other hand, inducible NOS import occurs in inflammatory conditions like experimental sepsis. The entrance of iNOS into mitochondria is more related to marked disruption of mitochondrial network resulting from decreased fusion-related proteins (mitofusin2) than to post-translational modifications. It is shown here, that modulation of NOS traffic into mitochondria plays a significant role in mitochondrial metabolic control or participates in the progression of inflammation to multiple organ damage.

P18.- EVALUATION OF ANTI-APOPTOTIC PATHWAY IN Bcr-abl +/- ACUTE LYMPHOBLASTIC CELLS

Lezama Palacios, Ruth Angélica; Dominguez Lopez, María Lilia; Montiel Cervantes, Laura Arcelia; Reyes Maldonado, Elba; Vasquez Franco, José Erasmo. ESCUELA NACIONAL DE CIENCIAS BIOLÓGICAS, IPN.

ralezama@hotmail.com

The acute lymphoblastic leukemia (ALL) is a lymphoid disorder that results from a proliferation and expansion of immature lymphoid cells. In 20-30% of adult patients present an unregulated kinase Bcr-abl. However patients who do not present this kinase have also a poor prognosis. We evaluate the PI3K/Src/NFKB activation pathway and the expression of Bcl-2 and c-IAP in Bcr-abl positive/negative cells from ALL patients and the relation of immunophenotype of these cells with the presence of Bcr-abl. The great majority of cases were Bcr-abl negative with immature immunophenotypes, this was related to high leukocyte count. We have observed a significantly activation of PI3K and Src kinase in Bcr-abl positive cells, and moderately activation of these kinases in Bcr-abl negative cells, in contrast NF-KB activation has been found in both Bcr-abl positive/negative cells. We found significative over expression of Bcl-2 and c-IAP in Bcr-abl positive/negative cells. These results suggest that Bcr-abl can contribute to activation of PI3K and Src, but is not essential in all ALL signaling pathways. Possibly, NFKB is involved in the Bcr-abl/PI3K/Src pathway in the case of Bcr-abl positive cells, but it could be activated by other kinases.

Posters

P19.-PHOSPHORYLATION DEPENDENT AND INDEPENDENT MECHANISMS IN REGULATION OF HISTAMINE H2 RECEPTOR BY GRK2

Alonso, Natalia¹ ; Alonso, Natalia² ; Gottardo, Federico² ; Monczor, Federico² ; Davio, Carlos² ; Fernandez, Natalia² ; Shayo, Carina¹. ¹ Instituto de Biología y Medicina Experimental - CONICET. ² Catedra de Química Medicinal - FFYB - UBA.

cshayo@dna.uba.ar

GRK2 mediated specific inhibition of GPCRs response usually involves receptor phosphorylation followed by arrestin binding and uncoupling from the G protein. However, GRK2-mediated GPCRs regulation also involves phosphorylation independent mechanisms. Here, we investigated whether the histamine H2 receptor (H2R), known to be desensitized by GRK2, needs to be phosphorylated for its desensitization, internalization and resensitization. We evaluated the effect of phosphorylating deficient GRK2K220R mutant on H2R signaling. We found that although this mutant functioned as dominant negative concerning receptor internalization and resensitization, it desensitized H2R signaling in the same degree as the GRK2 wild type. In order to identify the domains responsible for the kinase independent receptor desensitization, we cotransfected the receptor with constructions encoding the GRK2 RGS-homology domain (RH), and the RH or the kinase domain fused to the plekstrin homology domain. Only RH containing constructions desensitized the H2R. Overall, these results indicate that GRK2 induced desensitization of H2R through a phosphorylation independent, RGS dependent mechanism, although kinase activity proved to be necessary for receptor internalization and the resulting resensitization.

P20.-NITRIC OXIDE INDUCES HO-1 IN ADRENOCORTICAL CELLS

Astort, Francisco¹ ; Mercau, María¹ ; Martinez Calejman, Camila¹ ; Repetto, Martín¹ ; Arias, Pablo¹ ; Cymeryng, Cora B.¹. ¹ Departments of Physiology and Human Biochemistry, School of Medicine, University of Buenos Aires, CEFYBO-CONICET, Buenos Aires, Argentina.

f_Astort_lem@hotmail.com

Previous results from our laboratory demonstrated the reciprocal regulation of two local modulatory systems of adrenal steroidogenesis, e.g. nitric oxide (NO) synthase and heme oxygenase (HO). We then showed that adrenal HO-1 expression levels were positively regulated by endogenously generated NO. Present experiments were designed to analyze the mechanisms involved in the induction of HO-1 by NO in adrenal cells. Both HO-1 mRNA and protein levels were upregulated in Y1 cells incubated for 8 hours in the presence of a NO donor (DETA-NO, 1 mM). The signalling pathways involved were then analyzed. In regard to the NF κ B pathway, DETA-NO treatment resulted in a significant inhibition of the activity of the reporter plasmid KB-LUC, while overexpression of p65 decreased HO-1 protein levels. At the same time, DETA-NO had no significant effect on the activity of a Nrf2 reporter plasmid although Nrf2 overexpression significantly increased HO-1 protein levels. PKC inhibition blocked the increase in HO-1 protein levels triggered by the NO-donor. We hypothesize that in Y1 adrenal cells the NO-induced increase in HO-1 expression levels is negatively regulated by NF κ B-dependent mechanisms, is independent of the activation of the redox dependent transcription factor Nrf2, and is mediated by a PKC-dependent

P21.-PI3K SIGNALING AXIS IMPACT ON GH3 CELL INVASION OVER DIFFERENT EXTRACELLULAR MATRIX PROTEINS

Azor, Erika Patricia Oral and Pharyngeal Cancer Branch, NIDCR, NIH, USA.

ticuin@fisio.cinvestav.mx

Taking into account that integrins transmit chemical signals providing information on cell location, local environment, adhesive state, and that these signals determine cellular responses such as survival, differentiation and motility. We examined signaling pathways that leads to GH3 rat pituitary tumor cells to invade as a function of the context defined by surrounding matrix. We have previously report that cell morphology, adhesion, cytoskeleton array, hormone secretion and growth factor responses changes depending on the ECM component used in the culture. In the present work, we measured the invasion rate, analyzed the composition of the focal contacts and the activation state of their components to elucidate signaling pathway that modulates GH3 cell invasion over CI and CIV. Interestingly, we found that normally GH3 cell invasion is down regulated by the activity of a PI3K/PDK1/PKC/vinculin signaling pathway.

P22.-INTERACTION OF PKA WITH ITS REGULATED TARGET GENES IN RESPONSE TO STRESS CONDITIONS

Baccarini, Leticia¹; Fernando, Martinez Montañes²; Silvia, Moreno¹ ; Markus, Proff²; Paula, Portela¹. ¹Laboratorio de Biología Molecular y Transducción de Señales- Dpto de Química Biológica-FCEN-UBA. Argentina. ²Instituto de Biología Molecular y Celular de Plantas. Universidad Politécnica de Valencia. España.

lbaccarini@qb.fcen.uba.ar

Regulation of gene expression by intracellular stimulus-activated protein kinases is essential for cell adaptation to environmental changes. Previously, has been described that protein kinase are physically associated to the target gene. There are three PKA catalytic subunits in *S.cerevisiae*: Tpk1, Tpk2 and Tpk3 and one regulatory subunit: Bcy1. Using ChIP-real time assay we analyzed the Bcy1, Tpk1 and Tpk2 association to 10 genes regions in response to osmotic and oxidative stresses. We found that Tpk1 and Tpk2 but not Bcy1, were found associated with transcribed regions in glucose exponentially growing cells. Upon hyperosmotic stress we detected Bcy1 association at the ORF regions with concomitant Tpk2 and Tpk1 occupancy. Differentially, we observed Tpk1 and Tpk2 dissociation after oxidative stress at the promoter regions. We did not find Bcy1 associated to chromatin in response to oxidative stress. We characterize the role of PKA on gene expression; our results suggest that PKA activity, controlled through Bcy1, is direct or indirectly involved in the transcriptional control upon stress. These findings suggest that PKA could exert a transcriptional regulation by direct (or indirect) association to chromatin in stress and in a gene region-dependent manner.

**P23.-CDM EXHIBITS IMMUNOMODULATORY AND ANTIANGIOGENIC EFFECTS
WITHOUT AFFECTING NFkB AND p38MAPK PATHWAYS**

Bueno, Carlos¹; Barquero, Andrea¹; Maier, Marta²; Alché, Laura. ¹Laboratorio de Virología: agentes antivirales y citoprotectores, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. ²UMYFOR (CONICET-UBA) y Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

cbueno@qb.fcen.uba.ar

The antiviral limonoid 1-cinnamoyl-3,11-dihydroxymeliacarpin (CDM) modulates IL-6 and TNF- α production in macrophages stimulated with LPS or infected with HSV-1. CDM inhibits the vascular endothelial growth factor (VEGF) transcription as a consequence of the reduction of IL-6 level, but it does not prevent NF- κ B translocation in LPS stimulated-macrophages. These findings suggest that CDM could be affecting NF- κ B activation or an alternative cell signaling pathway involved in the production of cytokines. Our studies showed that CDM did not impede NF- κ B activation when macrophages were transfected with a NF- κ B-luciferase reporter plasmid and phosphorylation of p38 MAPK by Western blotting. Considering that CDM inhibits the transcription of VEGF, we analyzed the antiangiogenic properties of CDM on Human umbilical vein endothelial cell (HUVEC) formation of capillary-like tubes. CDM led to a drastic reduction in the number of capillary-like structures formation in a dose-dependent manner. In addition, this inhibition of HUVEC angiogenesis by CDM seemed to be a consequence of the modulation of IL-6 and TNF- α secretion. We conclude that the antiangiogenic activity of CDM would be a consequence of its immunomodulatory activity, affecting an alternative NF- κ B and p38 MAPK cell signaling pathway.

P24.-QUANTITATIVE MEASUREMENT OF SCAFFOLD PROTEIN MEMBRANE RECRUITMENT IN A GPCR SYSTEM IN YEAST

Bush, Alan¹; Chernomoretz, Ariel²; Colman-Lerner, Alejandro¹. ¹IFIByNE-UBA/CONICET. ² Departamento de Física, FCEN, UBA.

alerner2@gmail.com

The yeast (*Saccharomyces cerevisiae*) pheromone response pathway is one of the best understood G protein signal transduction systems. The dose response (DoR) curves measured at various levels of this pathway show a good alignment (i.e. similar EC50 values), a phenomenon that helps maintain fidelity in the information transmission and, though apparently intuitive, is not easily explained from the underlying highly non-linear biochemistry. Furthermore, this alignment depends on the activity of key components of the pathway, as Fus3MAPK and the GTPase Activating Protein Sst2GAP. In this work we measured the recruitment dynamics of the scaffold protein Ste5 to the membrane in a quantitative manner. This DoR curve is partially aligned with the receptor occupancy and transcriptional induction, but reaches steady state before the receptor equilibrates with its ligand. Mathematical modeling of the system suggests that a pheromone dependent activation of Sst2GAP might account for this dynamic behavior.

P25.-Rabs IN SPERM EXOCYTOSIS

Bustos, Matías¹; Rodríguez, Juan Facundo¹; Branham, María Teresita¹; Tomes, Claudia¹. ¹ IHEM-CONICET, School of Medicine, Cuyo National University.

ctomes@fcm.uncu.edu.ar

Rab3 and Rab27 dock secretory vesicles to the plasma membrane during exocytosis. Rab3 binds GTP and associates with vesicles; it later detaches upon GTP hydrolysis. Rab27 activation and targeting differ from those of Rab3. Exocytosis of sperm's secretory granule (the AR) is essential for fertilization; it relies on the same fusion machinery described in all cells plus cyclic AMP and Epac upstream the Rabs described above. Active Rab3A has a positive early role during the AR in human sperm. These cells contain an activity that exchanges GDP for GTP on Rab3A in response to AR triggers; the latter also enhance association of Rab3A with membranes. Surprisingly, recombinant Rab3A added at a late stage halts exocytosis if loaded with GTP- α -S but not with GTP, indicating that hydrolysis is necessary to bring exocytosis to completion. The exocytosis block is relieved by wild type α -SNAP but not by an inactive mutant, pointing to an unsuspected link between Rab3 and SNARE complex dissociation. Rab27 localizes to the acrosomal region in human sperm and it associates with membranes in both resting and stimulated cells. Rab27 exhibits an early role during exocytosis: is required for the docking of the acrosome to the plasma membrane and, surprisingly, for SNARE complex dissociation.

P26.-PHOSPHORYLATION OF Acyl-COA SYNTHETASE 4 (ACSL4) IN STEROIDOGENIC CELLS

Castilla, Rocío¹; Smith, Emilia¹; Podestá, Ernesto¹. ¹IIMHNO and Department of Biochemistry, School of Medicine.

rocio_castilla@yahoo.com

Acsl4, an arachidonic acid (AA) preferring enzyme, is key in the regulation of AA intracellular levels and steroidogenesis. Acsl4 is a protein with a high turn over rate, located in mitochondria-associated membranes (MAM) that acts in a dimer form. Steroid synthesis is regulated by different hormones or factors through PKA and/or PKC-mediated protein phosphorylation. Sequence analysis of Acsl4 shows the presence of PKA and PKC consensus sites. Thus, the aim of this study was to determine whether Acsl4 is substrate of these kinases. We demonstrated that recombinant Acsl4 is independently phosphorylated in vitro by PKA and PKC kinases. We also demonstrated that Acsl4 is phosphorylated by adrenocorticotrophin hormone in Y1 adrenal cells. The phosphorylation of Acsl4 does not regulate dimer formation but it increases the activity of the enzyme. Protein phosphorylation is essential for MAM/mitochondria association. Since Acsl4 participate in this association, Acsl4 phosphorylation may play a role in this mechanism.

P27.-TO ENTRAIN OR NOT TO ENTRAIN: THE NO –cGMP-PKG PATHWAY IN THE SUPRACHIASMATIC NUCLEUS

Chiesa, Juan J.¹; Plano, Santiago A.¹; Golombek, Diego A.¹; Baidanoff, Fernando M.¹.

¹Universidad Nacional de Quilmes /CONICET, Argentina.

jjosechiesa@unq.edu.ar

The NO/GC/cGMP/PKG pathway is essential for photic synchronization of the circadian clock. Phosphodiesterases (PDEs) are key regulators of intracellular cyclic nucleotide concentrations. To study the regulation of cGMP levels in the hamster SCN, we have determined by RT-PCR the presence of PDE5 and other isoforms in this model. In hamsters receiving specific PDE5 inhibitors (sildenafil, vardenafil or taladafil), reentrainment to a 6h phase-advance of the LD cycle took significantly shorter than controls. PDE5 inhibitors also elicited an increase in light-induced phase advances when injected 45 min before light stimulation at CT18. We have studied the role of NO in the intercellular communication within the dorsal and ventral portions of the SCN. Administration of the NO scavenger PTIO blocked photic phase advances and inhibited light-induced cFos-ir, without affecting phase delays. In addition, hamsters receiving a single dose of PTIO before light stimulation show an inhibition in the non-parametric entrainment to 23.5 h cycle and an inhibition of the light-induced Per1-ir. These results demonstrate that pharmacological inhibition of PDE5 affects photic entrainment, indicating a potential benefit for circadian disorders which require an increase in light signaling to the clock.

P28.-INSULIN RECEPTOR DYNAMICS BY A NOVEL DOMINANT-NEGATIVE RECOMBINANT RECEPTOR

Coluccio Leskow, Federico¹; Giudice, Jimena^{1,2}; Arndt-Jovin, Donna J.³; Jovin, Thomas M.³; Jares-Erijman, Elizabeth A.². ¹Department of Biological Chemistry, FCEN, UBA, Argentina. ² UBA-CIHIDECAR, CONICET, Argentina. ³Max Planck Institute for Biophysical Chemistry, Germany.

federico@fbmc.fcen.uba.ar

Insulin regulates different cellular processes such as transport and metabolism of glucose, lipids and proteins, nucleic acid synthesis and gene expression. Two splice variants of IR exist in mammals: IR-A lacking exon 11, and the full length IR-B. Both isoforms behave differently in the presence of ligands and cannot be distinguished by antibodies. While IR-A is expressed during development and has been shown to be misregulated in certain cancers, IR-B is expressed predominantly in metabolic tissues. The insertion of an ACP-tag in the extracellular domain of IR allowed us to label it in the membrane in living cells with small dyes and quantum dots. Recombinant receptor IR-B-A1x3 behaved as a dominant negative, binding insulin but not being capable of activating downstream signaling. This dominant negative IR (dn-IR) permits us to study the differential IR isoform dynamics and to gain insight into the controversial existence of heterodimers IR-A/IR-B in a new way.

**P29.-ARACHIDONIC ACID, A NOVEL REGULATOR OF GLI1 ONCOGENIC ACTIVITY
IN HUMAN CANCER CELLS**

Comba, Andrea¹; Pasqualini, Maria Eugenia¹; Vara Messler, Marianela¹; Das, Undurti N²; Valentich, Mirta Ana¹; Eynard, Aldo Renato ; Fernandez-Zapico, Martin E³. ¹Ira Cát. de Biol. Cel, Hist y Embr. IBC, FCM, UNC, Córdoba Argentina. ²School of Biotech, J. Nehru Tech. University, Kakinada, India. ³Schulze Center for Novel Therapeutics, Mayo Clinic, Rochester, MN USA.

andreacomba@hotmail.com

Increase expression of the zinc finger transcription factor GLI1 can lead to tumor development in different tissues. GLI1 overexpression is associated with poor survival and an aggressive phenotype in patients. Although these biological events are well described, the mechanism(s) controlling GLI1 expression in tumor cells remain elusive. Here, we identified a novel mechanism mediated by the araquidonic acid (AA) modulating GLI1 expression in cancer cells. AA is essential fatty acid with known tumor suppressor functions. In vitro studies using breast and pancreatic cancer cell lines exogenous AA reduced in a dose dependent manner, GLI1 transcriptional activity ($p < 0,05$). Analysis of the mechanism revealed that AA inhibits GLI1 promoter activity, thus, suggesting that this regulatory mechanism is through the modulation of GLI1 expression. Finally, owing the GLI1 pro-survival functions, we examine if AA influence this cellular function in GLI1-overexpressing cells. Interestingly, AA, significant decreased cell viability ($p < 0,05$) and increased apoptosis in these cancer cells ($p < 0,05$). Taken together, these results provide a novel insight into the complex signaling pathways involved in carcinogenesis and suggest a novel anti-tumor function of AA through the regulation of GLI1.

P30.-cAMP-DEPENDENT ACTIVATION OF STEROIDOGENESIS INVOLVES THE ACTION OF THE TYROSINE PHOSPHATASE SHP2

Cooke, Mariana¹; Maloberti, Paula¹; Duarte, Alejandra¹; Orlando, Ulises¹; Podestá, Ernesto¹; Cornejo Maciel, Fabiana¹. ¹IIMHNO and Department of Biochemistry, School of Medicine.

mcooke@fmed.uba.ar

Protein tyrosine phosphatases (PTP) play significant roles in many biological processes. PTP activation is a crucial step in the signal transduction cascade that leads to the activation of steroidogenesis triggered by ACTH and LH in adrenal cortex and Leydig cells respectively. We demonstrated that this cAMP-dependent protein tyrosine dephosphorylation is required to induce acyl-CoA synthetase 4 (Acsl4), which participates in the compartmentalized generation of the arachidonic acid (AA) needed for StAR (Steroidogenic Acute Regulatory) induction. StAR is a protein that participates in cholesterol transport between the mitochondrial membranes. Since SHP2 is a PTP activated by PKA phosphorylation in steroidogenic cells and it is related to Acsl4 induction and AA generation in tumorigenic processes, we tested here whether SHP2 is the PTP involved in the regulation of steroidogenesis. Using plasmid-mediated gene transfer and RNAi-mediated gene silencing we found that SHP2 expression level in MA-10 Leydig cells correlates with cAMP-stimulated production of progesterone, StAR protein levels and Acsl4 induction. Thus, we provide evidence for the first time that SHP2 participates in the cAMP-dependent signal transduction pathway that activates steroid production.

**P31.-MRP4 REGULATES cAMP LEVELS AND CONTROLS LEUKEMIA CELL
PROLIFERATION AND DIFFERENTIATION**

Copsel, Sabrina¹; Copsel, Sabrina²; Garcia, Corina¹; Diez, Federico¹ ; Vermeulem, Monica³; Bianciotti, Liliana⁴; Russel, Frans G.M.⁵; Shayo, Carina²; Davio, Carlos¹.

¹Catedra de Quimica Medicinal-FFYB-UBA. ²Instituto de Biologia y Medicina Experimental - CONICET. ³Departamento de Inmunología, Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina. ⁴Cátedra de Fisiopatología-FFYB-UBA.

⁵Department of Pharmacology and Toxicology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

cardavio@ffyb.uba.ar

Increased intracellular cAMP concentration play a well established role in leukemic cell maturation. We previously reported that U937 cells stimulated by histamine H2 receptor agonists, despite a robust increase in cAMP, fail to mature because of rapid H2 receptor desensitization and phosphodiesterase (PDE) activation. Here we show that intracellular cAMP levels in different human AML cell lines are also regulated by multidrug resistance-associated proteins (MRPs), particularly MRP4. U937, HL-60 and KG-1a cells, exposed to amthamine (H2 receptor agonist), augmented intracellular cAMP concentration with a concomitant increase in the efflux. Extrusion of cAMP was ATP-dependent and probenecid-sensitive, supporting that the transport was MRP mediated. Amthamine stimulation, combined with PDE4 and MRP inhibition, induced maximal cell arrest proliferation. Knock-down strategy by shRNA revealed that this process was mediated by MRP4. Furthermore blockade by probenecid or MRP4 knock-down showed that increased intracellular cAMP levels induce maturation in U937 cells. These findings confirm the key role of intracellular cAMP levels in leukemic cell maturation and provide the first evidence that MRP4 may represent a new potential target for leukemia differentiation therapy.

**P32.-RGDS INDUCES PROTEIN TYROSINE PHOSPHORYLATION WITHOUT
ACTIVATION IN *Bufo arenarum* (AMPHIBIA) OOCYTES**

Coux, Gabriela; Mouguelar, Valeria S. IBR (UNR-CONICET). Fac. de Cs. Bioq. y Farmacéuticas. UNR, Rosario.

coux@ibr.gov.ar

Integrins are cell adhesion molecules that are thought to be involved in sperm-oocyte interaction. In order to understand the role of integrin homologs in *Bufo arenarum* oocytes and their possible contribution to egg activation mechanisms, we examined the presence of integrin subunits and the effect of RGDS peptide on oocytes and during fertilization. Western-blot studies detected integrin subunits $\alpha 5$ and $\beta 1$ in oocytes but not in sperm. We found that RGDS peptide was unable to elicit egg activation or mitogen-activated protein kinase dephosphorylation, but produced reversible inhibition of fertilization. A similar partial inhibition was produced by an anti- $\beta 1$ integrin antibody. Using an anti-phosphotyrosine antibody we found major changes in phosphotyrosine containing proteins in egg extracts minutes after fertilization. Cytosol and plasma membranes isolated from oocytes and fertilized eggs showed additional fertilization-induced phosphorylated proteins. Some of these were also present in cytosol and plasma membranes from RGDS-treated oocytes (partially mimicking fertilization). These findings suggest that *B. arenarum* fertilization involves integrins (e.g. $\beta 1$ subunit) as adhesion proteins. Our data support the view that RGDS binding receptors may function as signaling receptors in *B. arenarum* oocytes, but integrin engagement by RGDS is not sufficient for oocyte activation.

**P33.-EXTERNAL CONTROL OF INTERNAL MOVEMENT: VESICLE TRAFFICKING
REGULATION BY HETEROTRIMERIC G PROTEINS**

Díaz Añel, Alberto Marcelo¹; Coria, Andrea Soledad¹; Masseroni, María Luján¹ ¹Instituto de Investigación Médica Mercedes y Martín Ferreyra.

adiazanel@immf.uncor.edu

Until a few years ago, it was thought that generation of transport carriers at the Trans-Golgi Network (TGN) had no regulation, hence its definition as “constitutive trafficking”. In our laboratory we have described a new trafficking regulatory pathway, required to manage protein secretion accordingly to the cell requirements. A key member of this signal transduction pathway is Protein Kinase D1 (PKD1). This kinase, when activated, is capable of translocate to TGN in order to regulate vesicle fission through the phosphorylation of currently unknown effectors. We have found that PKD1-dependent trafficking regulation is initiated at the plasma membrane by members of heterotrimeric GTP-binding proteins, particularly a subunits of Gq family and two specific bg subunits combinations, b1g2 and b3g2. Both G proteins subunits, a and bg, activate Phospholipases Cb (PLCb), which produce Diacylglycerol (DAG), that recruits PKD1 to the TGN and activates members of the novel Protein Kinase C (nPKC) subfamily. These PKCs phosphorylate PKD1 at the TGN, thus initiating membrane fission. New challenges arise from these results in order to complete this pathway, among them the characterization of G Protein Coupled Receptors linking external signals with intracellular protein trafficking regulation.

P34.-GH MODULATION OF EGF-INDUCED PI3K-AKT SIGNAL IN MOUSE LIVER

Díaz, María Eugenia¹; González, Lorena¹; Miquet, Johanna G.¹; Martínez, Carolina S.¹; Sotelo, Ana I.¹; Turyn, Daniel¹. ¹ IQUIFIB (UBA-CONICET), Pharmacy and Biochemistry Faculty, Buenos Aires, Argentina.

m_euge_diaz@hotmail.com

Epidermal growth factor receptor (EGFR) and growth hormone (GH) have been related to hepatocellular carcinoma development. Taking into account that the PI3K-Akt pathway is involved in tumorigenesis and the EGFR is transactivated by GH, the GH modulation of EGF-induced PI3K-Akt pathway was studied in liver. With this purpose, protein content and phosphorylation levels of PI3K-Akt pathway mediators were studied in the liver of transgenic mice that overexpress GH and in their normal siblings. Transgenic mice had a diminished response to EGF stimulation: Akt activation was reduced and its substrates, GSK3 and mTOR, were not phosphorylated even when they were overexpressed. This desensitization was not associated to diminished activity of PDK1, a kinase involved in Akt activation, or to hyperactivation of PTEN, a phosphatase involved in PI3K-Akt pathway inhibition. In contrast, overexpressed GH was associated with the reduction of Gab1 levels, a docking protein that allows PI3K activation, which might explain the decreased EGF-induction of the PI3K-Akt pathway. Moreover, increased SHP2 in membranes from transgenic mice and association of this phosphatase to Gab1 could result in the reduced activation of Akt observed in the transgenic mice.

P35.-HEAT SHOCK PROTEINS OF 60kDa FROM *K. pneumoniae* AND *E. COLI* IN APOPTOTIC DEATH OF MONONUCLEAR CELLS

Dominguez-López, María Lilia¹; Ortega-Ortega, Yolanda¹; Lezama-Palacios, Ruth Angélica¹; Dzul-Caamal, Ricardo¹; García-Latorre, Ethel Awilda¹; Vega-López, Armando¹. ¹ESCUELA NACIONAL DE CIENCIAS BIOLÓGICAS. IPN. PROL. DE CARPIO Y PLAN DE AYALA. CASCO DE SANTO TOMAS. MÉXICO D.F CP. 11340.

ldmguez@yahoo.com.mx

Heat shock proteins (HSPs) are highly conserved molecules. HSPs have been shown to have an anti-apoptotic role. We observed that enterobacterial HSP60 have anti-apoptotic effect on induced apoptosis by dexametasone (DXM) in peripheral blood mononuclear cells (PBMC) from healthy subjects (HS). Therefore, we investigated the effect of HSP60 from *K. pneumoniae* (HSP60Kp) and *E. coli* (HSP60Ec) on DXM induced apoptosis in PBMC from HS, and explored if this effect required protein internalization by the cell. The apoptosis was measured by peak G0 assay by flow cytometry. For assays on the anti-apoptotic effect of proteins, PBMC were pre-incubated with each protein before DXM addition. Results of apoptosis without and with DXM were 5.68% and 24.63%, respectively. The apoptotic percentage obtained with DXM was significantly reduced to 17.86 and 17.04% when the PBMC were pre-incubated with HSP60Kp and HSP60Ec, respectively. To evaluate the participation of cytoskeleton in this effect, the PBMC were incubated with or without nocodazole, cytochalasine D and amiloride. There was no reduction in the apoptosis when the PBMC were incubated with the inhibitors. These results suggest cytoskeleton participation in the anti-apoptotic effect showed by enterobacterial HSPs on apoptosis induced by DXM.

**P36.-PHOSPHORYLATION-INDUCED CONFORMATIONAL CHANGES IN Rap1b:
ALLOSTERIC EFFECTS ON EFFECTOR DOMAIN**

Edreira, Martín M.¹; Edreira, Martín M.²; Sheng, Li³; Ribeiro-Neto, Fernando²; Yeh, Joanne⁴; Buck, Matthias⁵; Woods, Jr, Virgil L.³; Altschuler, Daniel L.². ¹INGEBI-UBA-CONICET, Ciudad de Buenos Aires, Argentina. ²Department of Pharmacology and Chemical Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA. ³Department of Medicine and Biomedical Sciences Graduate Program, University of California, CA, USA. ⁴ Department of Structural Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA. ⁵ Physiology & Biophysics Department, Case Western Reserve Univ. Medical School, Cleveland, OH, USA.

mme2@pitt.edu

In models where cAMP exerts mitogenic responses, elevation of intracellular cAMP levels activates PKA and Epac, which synergistically stimulates Rap activation and phosphorylation at S179. It has been shown that the binding of GTP to Rap1b induces conformational changes at the switch I (residues 30-40) and the switch II (residues 60-76) domains, which are responsible for interaction with effectors. On the other hand, the role of phosphorylation is still unknown. Reports suggest that phosphorylation is able to modulate Rap1 association with some binding partners. The mechanism by which a modification at the carboxy-end of the molecule affects the regions involved with effector interaction at its N-terminus is for the moment unclear. We used amide hydrogen/deuterium exchange coupled with mass spectroscopy (DXMS), crystallography and NMR, to assess potential conformational changes induced by phosphorylation. Our results indicate that phosphorylation had no major impact on the secondary structure, but are consistent with an allosterically effect of the C-terminus to the switch loops/effector domain, that might act as an allosteric discriminator of conformational states.

P37.-TRANSLOCATION AND ACTIVATION OF nNOS INTO MITOCHONDRIA

Elguero, M. Eugenia¹; Poderoso, Juan José^{1,3}; Carreras, M. Cecilia^{1,2,3}. ¹Laboratory of Oxygen Metabolism, Hospital de Clínicas, University of Buenos Aires. ²Department of Biochemical Chemistry, School of Pharmacy and Biochemistry, University of Buenos Aires. ³CONICET.

carreras@ffyb.uba.ar

We previously reported increased mitochondrial translocation and activation of neuronal nitric oxide synthase (nNOS) in experimental hypothyroidism and by administration of insulin. In this study, we evaluated the contribution of Akt2 kinase and the Ca-dependent protease calpain1 in the process of nNOS import into mitochondria. By confocal microscopy, we observed the colocalization of nNOS in mitochondria after transient transfection of HEK293 cells with nNOS cDNA and in stably transfected HEK293 cells cotransfected with YFP vector bound to a mitochondrial target sequence. Either in vitro or in vivo (transcription-translation import assays or cell transfection), we observed a reduction of the apparent molecular weight of the protein from 157 to 130 kDa during import by the loss of the PDZ domain (determined by WB). In HEK293 cells pre-treated with insulin, activation of Akt2 increased nNOS translocation and phosphorylation in Ser1412 (C: 0.52 ± 0.04 vs I: 1.17 ± 0.31); no effects were observed using recombinant Akt. Activation of calpain by treatment with A23187 resulted in an increased mitochondrial expression of nNOS and the cleavage to the 130 kDa form. In conclusion, nNOS is imported and activated by a complex mechanism involving endogenous proteases, chaperones and kinases.

P38.-THE CALCITRIOL ANALOG EM1 HAS ANTINEOPLASTIC EFFECTS ASSOCIATED WITH VDR, p21 AND p27 UPREGULATION

Facchinetti, María¹; Salomón, Débora¹; Buschiazzo, Maximiliano¹; Mascaró, Evangelina²; Vitale, Cristian²; Radivoy, Gabriel²; Fall, Yagamare³; Curino, Alejandro¹.

¹Laboratorio de Biología Básica del Cáncer, INIBIBB-CONICET, Bahía Blanca, Argentina.

²Laboratorio de Química Orgánica, Departamento de Química, Universidad Nacional del Sur, Bahía Blanca, Argentina. ³Departamento de Química Orgánica, Facultad de Química, Universidad de Vigo, España.

facchinm@criba.edu.ar

The potent growth-inhibitory effects of calcitriol in several cell types make it an ideal compound to treat hyperproliferative disorders such as cancer. However, its hypercalcemic effects have severely hampered its therapeutic application and, to overcome this problem, structural analogs have been designed. In this work we analyzed the antineoplastic effects of the new analog EM1 on several human and murine tumor cell lines. We found a significant decrease in cell survival in glioma cells, an effect greater than that elicited by calcitriol. The reduction in cellular survival was accompanied by an increase in VDR, p21waf1/cip1 and p27kip1 and a decrease in cyclin D1, while p53 protein levels were not affected. These results were confirmed by qPCR. Moreover, EM1 induced p21 levels in glioma whereas calcitriol decreased it. Similarly, in a Kaposi sarcoma cell model (SVEC vGPCR), EM1 exerted antiproliferative effects accompanied by VDR and p27 up regulation whereas the non-malignant cells (SVEC) did not respond to it. Importantly, EM1 showed complete lack of calcemic activity in mice. These results suggest that EM1 is a promising analogue showing antiproliferative effects in glioma and Kaposi sarcoma associated with upregulation of VDR and the cell cycle inhibitors p21 and p27.

**P39.-BEHAVIORAL AND MOLECULAR CHARACTERIZATION OF A TRIPLE
TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE**

Feld, Mariana¹; Boccia, Mariano M.²; Blake, Mariano G.²; Krawczyk, María²; Casal, Josefina²; Baratti, Carlos M.²; Romano, Arturo G.¹. ¹Laboratorio de Neurobiología de la Memoria, Dto. Fisiología, Biología Molecular y Celular, Fac. Cs. Exactas y Naturales, UBA / IFIByNE, CONICET. ²Laboratorio de Neurofarmacología de los Procesos de Memoria, Cátedra de Farmacología, Fac. Farmacia y Bioquímica, UBA.

mfeld@fbmc.fcen.uba.ar

Alzheimer's disease (AD) is clinically characterized by progressive memory decline and cognitive dysfunction. Senile plaques containing mainly amyloid- β peptides, neurofibrillary tangles composed of hyperphosphorylated tau and neuronal loss are its major histological features. However, A β ; are elevated without plaque formation or nerve cell loss, yet learning and memory deficits are evident in early stages of AD. Transgenic mouse models of AD, such as the triple transgenic mice (3xTg) that progressively develop both amyloid and tangle neuropathology, are useful for elucidating factors that might affect the cognitive phenotype from incipient AD. Here we characterize the onset of learning and memory deficits at the age of 5 months in 3xTg mice, by using a Novel Object Recognition task (NOR). Concomitantly, we studied different molecular pathways in hippocampus (HIP) and prefrontal cortex (PFC) in order to establish potential early markers that likely correlate with the memory impairment observed. We found elevated ERK1/2 activity in PFC from 3xTg mice both extra-nuclear at 3 months and nuclear at 6 months of age. Taken together, our results suggest that ERK/MAPK pathway is early deregulated and would be partially responsible for the mild cognitive impairment observed in early stages of AD.

P40.-ERK PHOSPHORYLATION MECHANISMS INDUCED BY VASOPRESSIN IN VASCULAR SMOOTH MUSCLE CELLS

Gonzalez, Carlos B.; Villanueva, Carolina I.; Carmona, Pamela L. Department of Physiology, Universidad Austral de Chile, Valdivia, Chile.

cbgonzal@uach.cl

We have shown that vasopressin-induced up-regulation of the immediate early gene Egr-1 is carried out by transactivation of the EGFR. The up-regulation of Egr-1 is dependent upon the ERK activation. In the present work we show that vasopressin (AVP) is able to induce the ERK phosphorylation by EGFR-dependent and -independent pathways. We studied the ERK activation after AVP stimulation of A-10 cells, a cell line, derived from rat vascular smooth muscle cells, by Western blotting using phospho specific antibodies. In some experiments, prior the stimulation with AVP, cells were incubated with AG 1478, or with PP1 or Gö6983 inhibitors. AVP induced a biphasic ERK phosphorylation pattern; with an early peak response at 2.5- 5 min and a late response at 60-120 min. The early ERK phosphorylation response was partially inhibited by AG1478 an EGFR tyrosine kinase inhibitor. Accordingly, AG1478 also inhibited the early phosphorylation response of p90RSK, a downstream substrate of ERK. The early and the late AVP-induced ERK phosphorylation were sensitive to the pretreatment with the Src inhibitor PP1. Both ERK phosphorylation peak response were also sensitive to the pretreatment with Gö6983 a PKC inhibitor. These results suggest that the AVP-induced ERK phosphorylation by EGFR dependent and independent mechanisms. The Src activity appears to be required in both mechanisms. Moreover, the EGFR independent mechanism seems to involve the PKC activation. Supported by Fondecyt 1100871

P41.-GROWTH HORMONE (GH) SIGNALING IS DESENSITIZED IN COLON FROM TRANSGENIC MICE OVEREXPRESSING GH

González, Lorena; Gándola, Yamila; Miquet, Johanna G; Díaz, María Eugenia; Sotelo, Ana I; Turyn, Daniel. Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, (CP 1113), Buenos Aires, Argentina.

lgonza74@yahoo.com.ar

Transgenic mice overexpressing GH show increased growth of small bowel mucosa and reduced colonic apoptosis and frequently develop hepatocarcinoma at advanced ages. Livers from transgenic mice show increased expression of signaling molecules involved in cell proliferation and survival but response to GH and EGF (Epidermal Growth Factor) is diminished. The GH-IGF-1 system has important roles in intestinal homeostasis, growth and proliferation but the relevance of overexpressed GH for the development of colon cancer in mice is not clear. The objective of this study was to analyze the effects of GH excess on the expression and activation of signaling mediators involved in proliferation in colon. For this purpose, colon from GH-transgenic mice was studied. Although histological studies revealed polyps lesions in colon from transgenic mice, EGFR, Src, Akt, Erk1/2 and STAT5 expression and basal phosphorylation were found to be similar in normal and transgenic mice. However, GH-induced phosphorylation of STAT5 is diminished in the transgenic mice as well as EGF-induced phosphorylation of Akt. Desensitization of signaling pathways involved in cell proliferation and survival might be triggered in mice overexpressing GH to compensate exposure to high GH levels.

P42.-EGF-DEPENDENT BCL-X EXPRESSION IN MAMMARY EPITHELIAL CELLS

Grinman, Diego; Romorini, Leonardo; Coso, Omar ; Pecci, Adali. Dpto. Química biológica-FCEN-UBA. IFIBYNE-CONICET.

diegogrinman@qb.fcen.uba.ar

EGF activates signaling pathways associated with cellular proliferation and apoptosis. We have previously shown that the expression of the antiapoptotic protein Bcl-XL is regulated by EGF throughout apoptosis inhibition in HC11 mammary epithelial cells, being PI3K/AKT the main pathway involved in this process. EGF mediated bcl-X induction occurs mainly through the activation of bcl-X promoter P1, which contains CREB and ETS response elements, both plausible of being activated by the growth factor. In this work we analyzed the possible participation of these transcription factors on EGF dependent bcl-X expression. HC11 cells were co-transfected with a pP1-Luc reporter vector together with pAKT and the above mentioned transcription factors expression vectors, being CREB response the higher one. Co-transfections of P1-Luc and a CREB dominant negative expression vector confirmed that P1 as a CREB target. Moreover, EGF-dependent CREB phosphorylation was also observed. Conclusions: PI3K/AKT activation by EGF increases bcl-X levels by regulating P1, being CREB a potential transcription factor mediating this effect.

P43.-SET/I2PP2A PLAYS RELEVANT FUNCTION IN ORAL TUMORIGENESIS BY TRANSCRIPTION AND SIGNALING REGULATION

Leopoldino, Andreia¹; Garcia, Cristiana¹; Oliveira, Luciana¹; Pestana, Cezar²; Goto, Renata¹; Sayuri, Camila¹; Gutkind, Silvio³; Curti, Carlos². ¹Depto de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil. ² Depto Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil. ³ Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA.

andreiaml@usp.br

Set/I2PP2A protein is a potent inhibitor of phosphatase 2A. Recently we have identified it increased in oral squamous carcinoma cell (OSCC) samples. However its action in tumorigenesis and biological function is not understood yet. Our proposal was identification of new cell functions regulated by SET protein and its possible implication with OSCC. The strategies used were ectopic expression of SET in HEK293T cells and siRNA in OSCC cell line. The mRNA expression was investigated using quantitative PCR array system directed to 84 human transcription factors genes by real time PCR, also oxidative stress and detoxifying genes was evaluated. In signaling, its action was observed in p53, c-myc, Pten and Akt proteins. The SET action in PI3K-Akt signaling was showed using a model to oxidative stress-induced apoptosis and its effect in transcription was observed in oxidative defense genes, in detoxifying and in transcription factors genes. Specific SET regions showed different effect in Akt, c-myc and p53 levels. In conclusion, SET must act as oncogene in OSCC. Acknowledgments to Financial Support: CAPES, CNPq, FAPESP.

**P44.-GROWTH HORMONE SENSITIVITY IN MICE IS ASSOCIATED WITH CHANGES
IN SIGNAL TERMINATORS DURING GROWTH**

Martinez, Carolina S; Sotelo, Ana I; Turyn, Daniel; Piazza, Verónica G; Miquet, Johanna G. Instituto de Química y Físicoquímica Biológicas (IQUIFIB), UBA-CONICET, Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina.

carolinasmartinez@gmail.com

Growth hormone (GH) is an anabolic hormone that promotes longitudinal growth. Downregulation of GH signaling is achieved by induction of suppressors of cytokine signaling (SOCS), activation of tyrosine-phosphatases and internalization/degradation of GH-GHR complex. Somatic growth exhibits two instances of rapid growth in rodents: perinatally, independent of GH, and peripuberally, dependent on GH, at the third week of age. In order to assess negative modulators of GH signaling during growth, Swiss mice of three representative ages were chosen: lactating mice (1wk), mice showing major GH-response (2.5wk), and young adults (9wk). Protein levels were assayed by immunoblotting of liver extracts. Suppressors CIS and SOCS3 content was significantly higher in pups compared to young adults, the opposite was observed for SOCS2 (n=12, P<0.05). Phosphatases SHP1 and SHP2 abundance presented no age difference, while PTP1B displayed a profile similar to that of CIS and SOCS3. Although GH secretion is sexually dimorphic, no gender difference was observed for the proteins studied. We propose CIS, SOCS3 and PTP1B may modulate transition to GH-dependent growth by mitigating GH action before the onset of puberty, while SOCS2 may regulate GH action mainly in the young adult.

P45.-ANALYSES OF GENE EXPRESSION PROFILE IN JUVENILE NASOPHARYNGEAL ANGIOFIBROMA DEVELOPMENT

Martoreli da Silveira, Sara¹; Silva Wurzba, Sabrina Daniela¹; Brentani, Helena¹; Carraro, Dirce Maria¹; Soares, Fernando Augusto¹; Butugan, Ossamu²; Brentani, Maria Mitzi²; Rogatto, Silvia Regina¹; Rogatto, Silvia Regina³. ¹Department of Research, Antonio Prudente Foundation, AC Camargo Hospital, São Paulo, Brasil. ²University of São Paulo, São Paulo, São Paulo, São Paulo, Brazil. ³Department of Urology, FMB, UNESP - Sao Paulo State University, Botucatu, São Paulo, Brazil.

sara_martorelli@yahoo.com.br

Juvenile Nasopharyngeal Angiofibroma (JNA) is a locally invasive vascular tumor that occurs predominantly in male adolescents, suggesting that the tumor may be hormonally responsive. The expression profile of genes derived from tumoral biochemistry pathways (WNT, MAPK, PI3K pathways, and epithelial-mesenchymal transition genes-related), were evaluated by cDNA microarray, using a customized platform containing 2.3K genes in nine JNA. The gene expression profile resulted in 104 differentially expressed genes (80 down- and 24 up-regulated genes). The GOTM analysis (Gene Ontology Tree Machine website) demonstrated the involvement genes associated with colorectal cancer, focal adhesion, cell proliferation, WNT genes and neural development. Five genes (EDN1, LAMB1, RAF1, IGFBR4 and FTH), up-regulated were significantly associated with cell proliferation ($P=0.00098$). Thirteen genes (FTH1, AZGP1, CD74, IGF1, ING1, TGFB2, IGFB4, CDKNB, RAF1, LAMB1, EDN1, BCL2 and S100B) were involved in both, proliferation and neuron development. Overall, these data revealed potential markers involved in adhesion and proliferation processes in JNA. Neural development pathway-related genes could be a potential basis for improving current understanding of JNA development. Financial support: FAPESP, CNPq.

P46.-pERK EXPRESSION PATTERNS & OVERALL SURVIVAL IN NSCLC

Mendizábal, Javier Enrique¹; Galmés, Miguel A.²; Spizzamiglio, Nestor²; Mareso, Eduardo ³; Karo, Elina³; Hurtado Hoyo, Elías²; Gutkind, J. Silvio⁴; Coso, Omar¹. ¹ IFIBYNE-CONICET, Laboratorio de Fisiología y Biología Molecular, DFBMC-FCEN-UBA, Argentina. ²Hospital Carlos G. Durand/ARGENTINA. ³Facultad de Medicina, Universidad de Moron/ARGENTINA. ⁴Oral and Pharyngeal Cancer Branch NIDCR-NIH/USA.

jemendizabal@yahoo.com.ar

Erk1/2 (extracellular signal regulated kinases) are classical members of the MAPK (mitogen activated protein kinase) family that transduce extracellular signals activating nuclear transcription factors linked to cell proliferation and transformation. Among the deaths caused by cancer worldwide, 20% in Argentina, Lung Cancer is at the top. MAPK activity in NSCLC is still controversial. Using tissue microarray technology we studied by Immunohistochemistry the expression and subcellular localization of pErk in 355 lung cancer samples (253 NSCLC), and related it to gender, localization, histological subtypes, staging variables and overall survival. pErk was stained in NSCLC as in normal tissues including lungs. T1 tumors have intense (++++) stain, twelve times the value in the T2T3T4 p=0,009. While there were no differences in intensity stain between normal tissues and T1 samples it was significantly different between normal tissue and the T2T3T4 group p=0,005. T1 tumors show an expression pattern closer to the one present in normal tissues than to T2 tumors. Nuclear localization increases with stain intensity and is absent in normal samples. Patients with nuclear stain had a minor global survival than the rest p=0,02. Patients with higher stain intensity have a lower HR for death.

P47.-INDUCTION OF CYCLOOXYGENASE-2 (COX-2) BY LIPOPOLYSACCHARIDE (LPS) IN ADRENOCORTICAL Y1 CELLS

Mercau, María Elisa^{1,2}; Astort, Francisco^{1,2}; Martínez Calejman, Camila^{1,2}; Arias, Pablo³; Cymeryng, Cora Beatriz^{1,2}. ¹Laboratorio de Endocrinología Molecular, Dpto de Bioquímica Humana, FMED-UBA. ²CEFAYBO-CONICET. ³Depto de Fisiología, FMED-UBA.

mercaum@gmail.com

Previous studies from our laboratory demonstrate that LPS increases the production of glucocorticoids in a murine adrenocortical cell line (Y1). We have also shown that LPS increases the expression of COX-2, and hence the production of prostaglandins, potential modulators of adrenal steroidogenesis. In addition COX inhibitors have been shown to block the LPS-dependent increase in progesterone production. Present experiments were designed to analyze the signaling pathways involved in the regulation of COX-2 expression by LPS in Y1 cells. Our results show that LPS increases the activity of an NFκB reporter plasmid (KB-LUC) and that inhibition of this pathway blocked the stimulatory effect of LPS on COX-2 expression levels. In agreement, overexpression of p65 activates KB-LUC and upregulates COX-2 expression. In addition, LPS induces activation of p38 MAPK pathway and inhibition of this pathway blocked both KB-LUC activation and COX-2 induction by LPS. Based on these results we hypothesize that induction of COX-2 by LPS involves the activation of both NFκB and p38 MAPK signalling pathways and that activation of p38 MAPK might lead to NFκB translocation to the nucleus.

P48.-ROLE OF cAMP IN HISTAMINE-DEPENDENT REGULATION OF LEYDIG CELL PROLIFERATION AND STEROIDOGENESIS

Mondillo, Carolina¹; Pagotto, Romina¹; Monzón, Casandra¹; Besio Moreno, Marcos¹; Pignataro, Omar Pedro^{1,2}. ¹Laboratorio de Endocrinología Molecular y Transducción de Señales, Instituto de Biología y Medicina Experimental (IByME-CONICET). ²Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina.

caromondillo@hotmail.com

We recently reported a novel biological activity of histamine (HA) in the testis: the dual concentration-dependent regulation of Leydig cell (LC) steroidogenesis via H1 and H2 receptors. In this work we used the MA-10 cell line as an experimental model to evaluate the potential role of HA as modulator of LC proliferation, as well as its mechanism of action. We assessed cell proliferation by using [3H]-thymidine incorporation and MTS assays. Both HA and amthamine (A, H2 agonist) stimulated LC proliferation after 24 h. Given that H2 couples to the adenylate cyclase (AC) system in LC, we studied the effect of a 24-hour treatment with forskolin (FSK, direct stimulator of AC) or db-cAMP. Both compounds inhibited cell proliferation in a concentration-dependent manner. We then measured intracellular cAMP levels at various times after stimulation with HA, A or FSK. While HA and A raised cAMP levels transiently, FSK induced a sustained elevation. Interestingly, a 15-min treatment with db-cAMP stimulated cell proliferation, and the addition of a phosphodiesterase inhibitor 15 min after HA or A inhibited it. These results suggest that HA stimulates MA-10 LC proliferation via H2 activation and a transient increase in cAMP levels. Grants: ANPCYT, CONICET, UBA and F Roemmers.

P49.-ANALYSIS OF GENE EXPRESSION PROFILE OF INFLAMMATORY MOLECULES IN SOFT TISSUE SARCOMAS

Muto, Nair Hideko¹; Cunha, Isabela Werneck¹; Real, Juliana Monte¹; Dias, Adriana Abalen Martins¹; Reis, Luiz Fernando Lima².¹Hospital do Cancer AC Camargo, São Paulo, Brazil. ² Hospital Sírio Libanês, São Paulo, Brazil.

nair_muto@hotmail.com

Soft tissue tumors are a heterogeneous group of mesenchymal tumors that can be grouped according to biological behavior into three major categories, benign mesenchymal tumors (BMT), fibromatosis with local aggressiveness but no metastatic potential (DTF), and malignant sarcomas with metastatic potential (MMT). In a previous cDNA microarray study we compared the expression profile of BMT, DTF and MMTs and defined genes that are likely involved in local aggressiveness and metastatic potential, and also found some cytokines and interleukins as differentially expressed. In order to investigate the role of inflammatory molecules in this context, we performed a quantitative RT-PCR analysis of CXCL2, CXCL5, CXCL6, CX3CL1, CXCL11, IL-6, TNF α and PTX3 using BMT, DTF and MMT samples. Our results show no difference in CXCL2, CXCL11 and IL-6. The statistically significant differences were a higher expression of CX3CL1 and TNF α in MMT compared to DTF and a higher expression of TNF α in BMT compared to DTF. We also observed a tendency of higher expression of PTX3 and CXCL6 in BMT and a higher expression of CXCL5 in MMT. The next step will be to increase the panel of inflammatory genes and investigate the immunohistochemical expression of these molecules using a Tissue Microarray (TMA).

P50.- ALTERNATIVE MRNA EXPRESSION OF B1-INTEGRIN IN DIFFERENT STAGES OF MAMMARY GLAND DEVELOPMENT

Naipauer, Julian^{1,3}; Gattelli, Albana^{2,3}; LaMarre, Jonathan⁴; Degese, Sol^{1,3}; Vinuesa, Angeles^{1,3}; Coso, Omar^{1,3}; Kordon, Edith^{2,3}. ¹LFBM-DFBMC. ²LEGMA-DQB. ³IFIBYNE-CONICET-FCEN-UBA-ARGENTINA. ⁴DBMS, Ontario Veterinary College, University of Guelph, Canada.

juliannaipauer@gmail.com

Integrins are heterodimeric cell surface adhesion receptors that play a critical role in the normal development and tumor formation of the mammary gland. In this tissue, we observed that use of alternative polyadenylation sites produces a new b1-integrin (ItgB1) mRNA species, 578bp shorter than the one previously reported. The new species lack two AU-rich elements that might be implicated in mRNA stability. In fact, we observed that the longer 3'UTR, placed downstream of a CMV driven luciferase gene, produced a change in the stability of the corresponding mRNA. In the mouse, we found that different tissues and stages during mammary gland development show specific levels of each ItgB1 mRNA. For example, in this gland the longer form was up-regulated by estrogenic treatment and lactation. Similarly, in the HC11 mammary cell line, EGF treatment and *in vitro* differentiation specifically induced this species. On the other hand, post-lactation involution and HC11 cell stretching down-regulated this mRNA. Therefore, our data identify a new regulatory instance for controlling ItgB1 expression during mammary gland development and function, supporting other reports indicating that 3'UTR lengthen can be controlled by proliferation and differentiation states in embryonic and adult tissues.

P51.-21-HIDROXI-6,19-EPOXYPROGESTERONE: A LEAD COMPOUND WITH DISSOCIATED GLUCOCORTICOID ACTIVITIES

Orqueda, Andres¹; Presman, Diego¹; Alvarez, Lautaro²; Kordon, Edith¹; Burton, Gerardo²; Pecci, Adali¹. ¹Department of Biological Chemistry, FCEN-UBA and IFIBYNE-CONICET. ² Department of Organic Chemistry and UMYMFOR.CONICET.

aorqueda@qb.fcen.uba.ar

Glucocorticoids (GCs) exert their action throughout two main mechanisms: transactivation (gene expression induction by GR binding to specific GRE sequences) and transrepression (modulation of different transcription factors activities). In clinical trials beneficial effects of GCs are often associated with transrepression, while adverse effects are associated with transactivation. 21-hidroxi-6,19-epoxyprogesterone (OP) is a GR ligand postulated as a putative steroid regulator glucocorticoid modulator (SRGM). Here, we investigated OP properties as a dissociated glucocorticoid. We found that OP behaves as an agonist in transrepression assays, as it inhibits Rel A and AP-1 activities as well as TNF- α mediated cox-2 and IL-8 induction. On an opposite way, OP antagonizes GR mediated transactivation effects, as it inhibits bcl-XL expression and GR dependent MMTV-driven gene expression. Interestingly, OP does not inhibit GR nuclear homodimerization but prevents GR-TIF-2 coactivator recruitment. Finally, different from most commercial GCs, OP lacks the ability to inhibit apoptosis triggered by chemotherapeutics in mammary tumor cells. Thus, OP emerges as a novel SRGM with potential clinical application as it maintains antiinflammatory activities without inducing chemoresistance.

**P52.-EXPRESSION REGULATION OF C SUBUNIT OF PROTEIN KINASE A FROM
*Saccharomyces cerevisiae***

Pautasso, Constanza; Portela, Paula; Rossi, Silvia. Dpto. Química Biológica, FCEyN, UBA.

cpautasso@qb.fcen.uba.ar

One mechanism that yeast cells use to protect the internal system from the effects of environmental variation is to initiate a common gene expression program to protect the cell and many of these genes can be related to specific signaling pathways. The aim of this work is to study the expression regulation of two catalytic isoforms of protein kinase A, Tpk1 and Tpk3, in *S.cerevisiae*, under different conditions of stress and carbon source availability. The activity of TPK1 promoter is high in the diauxic-shift, while TPK3 promoter activity remains constant. In the presence of glycerol, both promoters activities are higher than in glucose presence. We demonstrated that TPK1 and TPK3 promoters activities are inhibited by PKA and this regulation is isoform-specific. Under heat shock and osmotic stress conditions, TPK1 promoter activity increases while TPK3 promoter activity remains constant. By in silico analysis and using different mutants we demonstrated that Msn2/4, stress transcription factors, regulate positively TPK1 but not TPK3 promoter activity. ChIP assays showed a decreased Msn2 occupancy on TPK1 promoter during heat shock, but increase of Pol II occupancy indicating that the promoter was transcriptionally active. Results are in agreement with "black widow" model.

P53.-ACTIVATION OF PKCalpha AND PKCepsilon INDUCES TUMORAL LACTOTROPH PROLIFERATION VIA ERK1/2

Petiti, Juan Pablo¹; Gutiérrez, Silvina¹; De Paul, Ana¹; Andreoli, Verónica²; Mukdsi, Jorge¹; Sosa, Liliana¹; Bocco, José Luis²; Torres, Alicia Inés¹. ¹Centro de Microscopía Electrónica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. ²CIBICI-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.

jpetiti@cmefcm.uncor.edu

We explored the role of PKCalpha and PKCepsilon as mediators of phorbol 12-myristate 13-acetate (PMA)-induced proliferation in pituitary tumor GH3B6 cells, and determined if the ERK1/2 and Akt pathways were activated. The GH3B6 cell proliferation was estimated by BrdU incorporation and the cell cycle progression by flow cytometric cell cycle analysis. We determined the expression of PKCalpha and PKCepsilon in membrane and cytosolic fractions by western blotting. The subcellular redistribution of both PKC isozymes was analyzed by confocal and immunogold electron microscopy. Incubation with PMA for 15 min stimulated PKCalpha and PKCepsilon activation, which was correlated with its translocation to plasma and nuclear membrane and phosphorylation of ERK1/2 but not Akt. The activation of both these PKC isozymes was closely associated with the stimulation of proliferation and the cell cycle progression induced by PMA, an effect that was blocked by the inhibitors of PKCalpha (Gö6976) and PKCepsilon (eV1-2). In addition, the pretreatment with the inhibitor of ERK1/2 (PD98059) prevented the mitogenic activity induced by PMA for 15 min. We demonstrated that the activation of PKCalpha and PKCepsilon by phorbol ester in tumor pituitary GH3B6 cells led to cell proliferation via ERK1/2.

P54.-MELATONIN AND LUZINDOLE ANTAGONIZE GLUCOCORTICOID RECEPTOR ACTIVITY BY DIFFERENT MECHANISMS

Presman, Diego^{1,2} ; Levi, Valeria¹; Pecci, Adali^{1,2}. ¹Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. ²IFIBYNE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

diegopres@qb.fcen.uba.ar

The antagonism exerted by the pineal hormone melatonin (MEL) on the glucocorticoid action has been well established; however, its molecular determinants remain still unknown. Previously, we had described that MEL inhibits glucocorticoid receptor (GR) nuclear translocation on mouse thymocytes, most likely by preventing GR-Hsp90 dissociation. Here, we show that in baby hamster kidney cells (BHK21), MEL neither affects GR translocation nor GR nuclear homodimerization, but rather impairs GR interaction with the transcriptional intermediary factor 2 (TIF2). The use of the Mel 1 receptor antagonist Luzindole curiously reveals that this molecule not only fails to block MEL effect but also has potential antiglucocorticoid properties per se. In fact, Luzindole is able to inhibit glucocorticoid-dependent MMTV-driven gene expression without affecting GR translocation or GR-TIF2 interaction. Together, these results reveal that both methoxyindoles antagonize GR activity by different mechanisms and reveal an unexpected diversity on the mechanisms by which MEL can block GR action.

**P55.-KLF4 EXPRESSION AND ABERRANT TRANSFORMING GROWTH FACTOR B
PATHWAY IN HEAD AND NECK CANCER**

*Raimondi, A. R.*¹; *Alvarez, R. S.*¹; *Sanchez, V.*¹; *Bal de Kier Joffé, E.*¹; *Gutkind, J. S.*².
¹ *Institute of Oncology Angel H. Roffo, University of Buenos Aires, Argentina.* ² *Oral and Pharyngeal Cancer Branch, NIDCR, NIH, USA.*

anaraimondi@conicet.gov.ar

Head and neck cancer (HNC) is among the six most prevalent cancers in the world. Despite improvements in treatment, survival has not changed over the past 30 years. KLF4 is a transcription factor associated with carcinogenesis and tumor suppression. KLF4 is expressed in differentiated cells of normal stratified epithelia. Here we analyze the expression of KLF4 in HNC and its possible association with TGF β pathway. We studied the basal expression levels of KLF4 in HNC cells by western blot. Increased level of KLF4 was detected in all cell lines compared with normal keratinocytes. After treatment of HNC lines with TGF β 1 we found reduced expression of KLF4 in HN13 cells. When we studied the viability of cells by MTS assays, none of the HNC cells showed growth inhibition by TGF β 1; however HN30 presented a partial reduction of 10%. To further understand TGF β resistance we studied the induction of pSmad2 and p21. HNC cells showed induction of pSmad2; no change was found in HN12 cells for p21. Overall, HNC cells presented a significant resistance to TGF β ; growth inhibitory effect. HNC cells retained smad2 activation and HN13 a regulation in KLF4 expression. The progressive disruption of TGF β signaling and the changes in KLF4 regulation may reflect different stages in oral tumorigenesis.

P56.-RUNX1 AND FOXP3 INTERACTION: POTENTIAL MECHANISM OF ONCOGENE REGULATION IN MAMMARY GLAND

Recouvreux, Maria Sol¹; Grasso, Esteban²; Echeverria, Pablo³; Castilla, Lucio⁴ ; Kordon, Edith¹; Rubinstein, Natalia¹. ¹LEGMA-IFIBYNE-CONICET, FCEN-UBA, Argentina. ²Laboratorio de Inmunofarmacología, QB, FCEN-UBA, Argentina. ³Departement de Biologie Cellulaire, Universidad de Ginebra, Suiza. ⁴UMASS, Boston, USA.

nrubinstein@fbmc.fcen.uba.ar

Previously, our laboratory reported the association of the RSPO3 gene and mammary tumorigenesis. In silico analysis of RSPO3 promoter sequence revealed 3 putative sites for Runx1 binding and none for Foxp3. In immune cells, Foxp3 inhibits Runx1 transcription factor activity. It was determined that normal mammary epithelial cells (NC) express Foxp3, which is absent in tumor cells (TC). The goal of this work was to evaluate the participation of Runx1 and Foxp3 in RSPO3 expression regulation on NC and TC. Using Western blot (WB) and qPCR analysis we did not find differences in Runx1 expression between NC and TC. By EMSA we determined that Runx1 binds to RSPO3 promoter and Runx1 overexpression resulted in RSPO3 enhanced expression. Co-immunoprecipitation assays showed that Runx1 and Foxp3 interact in NC. In normal mouse mammary gland we found that Runx1 and RSPO3 expression is induced during MG involution while Foxp3 is absent after pregnancy. To look for another Runx1 gene targets we performed omics data integration and mining using different computational approaches. This analysis revealed that Runx1 is a potential gene regulator in the promotion of MG tumors. These results suggest that Runx1 and Foxp3 interaction could be a potential mechanism of oncogene regulation in mammary gland.

P57.-SIGNAL TRANSDUCTION PATHWAYS IN THE REGULATION OF SERTOLI CELL PROLIFERATION

Riera, María Fernanda; Galardo, Maria Noel; Pellizzari, Eliana Herminia; Cigorraga, Selva Beatriz; Meroni, Silvina Beatriz. Centro de Investigaciones Endocrinológicas (CEDIE/CONICET).

smeroni@cedie.org.ar

Sertoli cells (SC) provide the structural and nutritional support for germ cell development. Considering that each SC is able to support a limited number of germ cells, the final number of SC reached during the proliferative periods determines sperm production capacity. FSH stimulates SC proliferation during fetal and neonatal periods. We analyzed transduction pathways involved in FSH stimulation of SC proliferation and those mechanisms that may be involved in cessation of proliferation at the time of blood-testis barrier formation. Cultures of mitotically active SC isolated from 8-day-old rats were used. We observed that FSH, in addition to PKA, stimulates PI3K/PKB and P70S6K signaling pathways and that all of them participate in SC proliferation. In addition, we observed that FSH inhibits MAPK and AMPK signaling pathways. On the other hand, stimulation of AMPK produced a decrease in FSH-stimulated SC mitosis concomitant with an increase in mRNA levels of the cell cycle inhibitor p19INK4. Altogether, these results suggest that several signal transduction pathways participate in the regulation by FSH of SC proliferation and that activation of AMPK is essential for the regulation of p19INK4d expression and for cessation of SC proliferation in the continuous presence of FSH.

P58-CYTOPROTECTIVE ROLE OF NRF2-ARE PATHWAY AND HSP70 IN NEONATAL UNILATERAL URETERAL OBSTRUCTION (UUO)

Rinaldi Tosi, Martin^{1,2} ; Bocanegra, Victoria¹; Manucha, Walter^{1,2}; Gil Lorenzo, Andrea¹; Vall, Patricia^{1,2}. ¹Laboratory of renal physiology and pathophysiology. Institute of Medicine and Experimental Biology of Cuyo (IMBECU-CONICET). Mendoza, Argentine. ² Pathophysiology Area. School of Medicine. (FCM-UNCuyo). Mendoza, Argentine.

rinaldi@lab.cricyt.edu.ar

We analyzed the Hsp70 response and the Nrf2 transcription factor signal transduction on UUO oxidative stress modulation. Rats were subjected to UUO or sham operation and kidneys harvested at 5, 7, 10 and 14 days after obstruction. Hsp70 and Nrf2 activity and its downstream target gene products were assessed. After 10 and 14 days of obstruction, enhanced lipid peroxidation through higher TBARS levels and reduced total antioxidant activity and enhanced NADPH oxidase activity were demonstrated. This was accompanied by decreased inducible Hsp70 expression and a progressive reduction of nuclear Nrf2 and its gene products GSTA2 and NQO1, whereas the Nrf2 repressor Keap1 was upregulated. By contrast, on early obstruction for 7 days; lack of increased oxidative markers associated with higher inducible Hsp70 protein levels and a rapid nuclear accumulation of Nrf2, Keap1 downregulation and mRNA induction of the identified Nrf2-dependent genes, NQO1 and GSTA2, were shown. We suggest that the cytoprotection in early obstruction depends on the combined contribution of induced activation of Nrf2 upregulating its downstream gene products and Hsp70 response. Impaired ability to mount the biological response to the prevailing oxidative stress leading to renal injury was shown in prolonged obstruction.

P59.-THE MAMMALIAN TARGET OF RAPAMYCIN (mTOR) IN THE SIGNALING PATHWAY OF Gbetagamma

Robles-Molina, Evelyn¹; Guzmán-Hernández, María Luisa¹; Reyes-Cruz, Guadalupe²; Vázquez-Prado, José¹. ¹Dept. of Pharmacology. CINVESTAV-IPN. ²Dept. of Cell Biology. CINVESTAV-IPN.

evelux07@hotmail.com

G-protein coupled receptors constitute the most abundant group of plasma membrane receptors and regulate multiple cellular functions. Upon receptor activation, G_α releases GDP, binds GTP, and dissociates from G_{βγ}, acquiring both, G_α-GTP and G_{βγ} the ability to interact with their respective effectors. G_{βγ} activates a growing list of effectors including RacGEFs and PI3K isoforms. GPCRs activate mTORC2, a multiprotein complex consisting of mTOR, Rictor, mSin1, Protor, and mLST8, leading to AKT phosphorylation and cell survival. However, the mechanisms by which GPCRs activate mTORC2 are still under discussion. We provide evidence indicating that mTORC2 is a critical participant in the signaling pathway leading to AKT activation and cell survival in response to sphingosine-1-phosphate. Using the yeast two hybrid system, we demonstrated an interaction between G_β and the carboxyl terminal region of mTOR, we confirmed this interaction in mammalian cells. Interestingly, mTOR shows affinity for distinct G_{βγ} heterodimers except G_{β4γ2}. Collectively, these results suggest the interesting possibility that G_{βγ} regulates mTORC2 via a direct interaction, opening an alternative link in a signaling pathway leading to the activation of AKT and its downstream targets.

**P60.-A NEW CONFORMATION OF THE MAPK ACTIVATION LOOP REVEALS THE
AUTOPHOSPHORYLATION MECHANISM**

Rodriguez Limardo, Ramiro G.^{1,2}; Ferreiro, Dardo N.^{1,2}; Marti, Marcelo A.^{1,2}; Turjanski, Adrian G.^{1,2}. ¹Departamento de Química Biológica, Universidad de Buenos Aires, Intendente Güiraldes 2160, Ciudad Universitaria, C1428EGA, Ciudad de Buenos Aires, Argentina. ² INQUIMAE, Universidad de Buenos Aires, Intendente Güiraldes 2160, Ciudad Universitaria, C1428EGA, Ciudad de Buenos Aires, Argentina.

ramiro_rl@hotmail.com

Mitogen-Activated Protein Kinases (MAPKs) are serin/threonin kinases which phosphorylate proteins with the S/T-P motif. MAPKs require double phosphorylation in two highly conserved tyrosine and threonin residues. In spite of the great knowledge that exists on MAPKs structure, little is known about the mechanism that regulates activation and interaction with other proteins because the static structures are very similar. In a previous study of the dynamics of one of this proteins, p38 γ (being its phosphorylation site TGY), a new spatial conformation not described before was found. Such conformation has a great stability in which the tyrosine seems to be close to the catalytic site and, as a consequence, it is accessible for its intramolecular auto-phosphorylation. We want to identify, by the The Jarzynski equality (JE), the free energy barriers between this structure and the ones already described (where the activation lip is exposed to the solvent) in order to compare them with the auto-activation data shown previously experimentally. Our results are in agreement with many experimental results and they will expand our knowledge on this kinases family.

P61.-KINETIC OF EXTRACELLULAR ATP ACCUMULATION FROM HYPOTONICALLY CHALLENGED HEPATOMA CELLS.

Sanchez Alberti, German¹; Alvarez, Cora²; Espelt, Maria Victoria³; Schwarzbaum, Pablo Julio⁴.

pjs@qb.ffyb.uba.ar

In most animal cells, release of cytosolic ATP into the extracellular space occurs in response to osmotic gradients, with either cAMP and/or Ca²⁺_i signaling such release. In this study, we evaluated the main processes governing the kinetic of extracellular ATP (ATPe) accumulation for Huh-7 hepatoma cells exposed to hypotonicity. Exposure of cells to a 180 mOsm hypotonic medium led to an exponential increase of [ATP]_e to a maximum (33.4 ± 11.33), followed by a slower non linear decrease ($t_{1/2}=11$ min). ATPe accumulation was inhibited 75% by carbenoxolone, an inhibitor of Pannexin 1, whereas pre-treatment of cells with the purinergic receptor P2Y₁₃ agonist 2-Methylthio adenosine 5'diphosphate (which inhibits adenylate cyclase) led to a 29% inhibition. Cells display ectoATPase activity ranging from 0.5×10^{-3} nmoles \times (sec \times 10⁶cells)⁻¹ (at 60.5 nM) to 4.9×10^{-3} nmoles \times (sec \times 10⁶cells)⁻¹ (at 970 nM). Since under these conditions cell mortality is negligible, the kinetic of ATPe is mainly controlled by ectoATPase activity (which consumes ATPe) and non lytic ATP efflux (which generates ATPe), with this latter efflux being partially inhibited by P2Y₁₃ activation and partially mediated by Pannexin 1. With grants from UBA, CONICET, and ANPCyT (PICT 1432).

P62.- NRF2 PROTEINS MEDIATE HO-1 EXPRESSION TRIGGERED BY VGPCR.

Sapochnik Daiana; Martin Maria Jose; Marinissen Maria Julia; Tanos Tamara and Coso Omar. Laboratorio de Fisiología y Biología Molecular. Facultad de Ciencias Exactas y Naturales, IFIBYNE UBA-CONICET, Buenos Aires, Argentina.

dsapochnik@fbmc.fcen.uba.ar

Heme oxygenase-1 (HO-1) is an enzyme upregulated by the Kaposi's sarcoma-associated herpesvirus (KSHV) and highly expressed in human Kaposi Sarcoma (KS) lesions. The oncogenic G protein-coupled receptor (KSHV-GPCR or vGPCR) is expressed by the viral genome in infected cells and is involved in KS development, HO-1 expression and vascular endothelial growth factor (VEGF) expression. We have characterized that vGPCR induces HO-1 expression and HO-1 dependent transformation through the $G\alpha_{13}$ subunit of heterotrimeric G proteins and the small GTPase RhoA. We are currently narrowing down the molecular components that regulate vGPCR triggered HO-1 expression at the promoter level. We found several lines of evidence that support a role for Nrf2 transcription factors and family members in the vGPCR- $G\alpha_{13}$ -RhoA signaling pathway. Different kinase pathways have already been shown activated in cells expressing vGPCR. Our current information assigns a major role to the Erk2 pathway as an intermediate in signaling from vGPCR to Nrf2. Altogether, our studies show that vGPCR induces HO-1 expression through signalling pathways that target the HO-1 promoter via Nrf2 transcription factors, broadening the range of molecular potential therapeutic targets for KS or tumors with high HO-1 activity.

P63.-ANG II AT2 RECEPTORS INVOLVE PARTICIPATION OF THE COMPLEX SHP-1/c-Src/FAK AND ERK1/2 ACTIVATION

Seguin, Leonardo; Ciuffo, Gladys; Blanco, Helga. IMIBIO-SL, CONICET. UNSL. Av. Ejército de los Andes 950 - SAN LUIS (5700).

lseguin@unsl.edu.ar

While Ang II effects mediated by AT1 receptors are well-known, the signal transduction and functions of AT2 receptors remain unclear. The aim of this study was to elucidate the non-classical signaling pathway of Ang II AT2 receptors in PND15 rat hindbrain, a critical developmental stage. Membrane preparations were stimulated with Ang II in the presence or not of antagonists, the c-Src inhibitor PP2 or its inactive analog PP3. Following solubilization, proteins were immunoprecipitated with anti-AT2 or anti-SHP-1 antibodies. Ang II stimulation promotes SHP-1 association to AT2 receptors selectively. Immunocomplexes obtained with anti-AT2 or anti-SHP-1 exhibited PTPase activity, blocked by PD123319 (AT2 antagonist) or PP2. c-Src was present in these immunocomplexes and PP2 blocked SHP-1 tyr-phosphorylation as well as activation and SHP-1/c-Src association. AT2 immunocomplexes also contained FAK kinase but not paxillin. Besides, AT2 receptor's activation induced activation of ERK1/2. Thus, Ang II AT2 receptors stimulation induced Tyr-phosphorylation of SHP-1 and immunocomplexes formation between AT2/SHP-1/c-Src/FAK. These results demonstrate an inducible signaling pathway of Ang II AT2 receptors suggesting a potential role in a critical stage of rat hindbrain development.

**P64.-TARGETED DELIVERY OF EGF-QDOTS COMPLEXES INTO RAT
HEPATOCYTES SUBJECTED TO HYPOTHERMIC PRESERVATION**

Sigot, Valeria¹; Rodr, Joaqu^{1,2} ; Pellegrino, Jos³; Guibert, Edgardo^{1,4}. ¹Centro Binacional (Argentina-Italia) de Criobiolog. ²Area Farmacolog. ³IFISE. Facultad de Ciencias Bioqu. ⁴Depto. Biolog.

vsigot@fbioyf.unr.edu.ar

Receptor mediated transport is an energy dependent mechanism requiring an available pool of ATP as well as the integrity of the cell membrane for efficient receptor activation and endocytosis. During cold storage of cells, a necessary step to preserve cells for transplant, both conditions are compromised. In order to evaluate the effect of hypothermic preservation on receptor-mediated transport, we monitored the targeted delivery of fluorescent Quantum Dots (QDs) conjugated to the Epidermal Growth Factor (EGF) ligand into cold-stored rat hepatocytes. The EGF-QDs complexes were added to cultured hepatocytes (CH) and to cold-stored hepatocytes (CSH) preserved in University of Wisconsin solution (UW) at 4°C (24 and 72 h). EGF-QDs were monitored in real time by confocal microscopy during re-warming to 37°C. After 30 min., QDs were internalized in CH and also in 24 h-CSH regardless of EGF-QDs complexes were added to the UW solution or prior to rewarming up to 37°C, whereas targeted QDs remained in the cell membrane of cells preserved in UW for 72 h. Negligible binding was observed for non-targeted QDs. These preliminary observations indicate that targeted QDs may act as suitable biosensors for cold-induced membrane injury and its effect on the EGF-receptor mediated transport.

P65.-BOAR SPERM AKAP4 IS TYROSINE-PHOSPHORYLATED BY SPERM BINDING GLYCOPROTEIN

Teijeiro, Juan Manuel¹; Marini, Patricia¹; Cane, Fernando². ¹Instituto de Biología Molecular y Celular de Rosario. CONICET. Facultad de Ciencias Bioquímicas y Farmacéuticas. Universidad Nacional de Rosario. ²Instituto de Porcinotecnia. Ministerio de la Producción. Provincia. Santa Fe.

teijeiro@ibr.gov.ar

During their journey in the female tract, sperm come into contact with epithelial cells that line the organs. Such interaction allows the selection of sperm with certain qualities. The pig oviductal glycoprotein Sperm Binding Glycoprotein (SBG) produces in vitro, acrosome alteration and the tyrosine-phosphorylation of a 97 kDa sperm protein (p97). The aim of this work was to identify this tyrosine-phosphorylated protein and gain some insight about its possible phosphorylation pathway. The outcome of LC-MS/MS analysis indicated that p97 contains peptides also present in canine AKAP4. A pig EST containing the same sequences was used for fusion protein expression. Antibodies generated against this protein were used for its immunolocalization on sperm and testicular tissue. The involvement of cAMP/PKA and PKC signaling pathways was studied using dbcAMP, IBMX and phorbol 12-myristate 13 acetate, correspondingly. Our results indicate that AKAP4 is phosphorylated during boar sperm-SBG interaction and that the PKC signaling pathway may be involved in this tyrosine-phosphorylation.

**P66.-CAVEOLIN-1 PROMOTES CELL POLARIZATION AND DIRECTIONAL
MIGRATION OF METASTATIC MELANOMA CELLS.**

Urra, Hery¹; Torres, Vicente¹; Lobos, Lorena¹; Ortiz, Rina¹; Díaz, Natalia¹; Härtel, Steffen²; Leyton, Lisette¹; Quest, Andrew¹. ¹Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), ICBM, Facultad de Medicina, Universidad de Chile. ²Anatomy and Developmental Biology Program, ICBM, Facultad de Medicina, Universidad de Chile.

aquest@med.uchile.cl

Objective: In mesenchymal cells, Caveolin-1 promotes directional cell migration in a manner that requires localization to the rear of migrating cells. Some reports associate presence of this protein with enhanced metastasis. Here Caveolin-1 participation in the migration of metastatic melanoma cells was evaluated. Methodology: Cell polarization and Caveolin-1 localization were assessed by immunofluorescence analysis of metastatic B16F10 melanoma cells transfected with either the vector placIOP (Mock) or pLacIOP-Caveolin-1 (Cav-1). Planar migration and focal adhesion turnover were evaluated by time-lapse video microscopy. 3D migration was studied using the Boyden chamber assay. Results: In B16F10 cells, expression of Caveolin-1 increased polarization, velocity and directionality of migration compared with Mock cells. Also, focal adhesion turnover and the number of transmigrating cells increased. Unlike in mesenchymal MEF-3T3 cells, Caveolin-1 did not localize to the trailing edge of B16F10 cells. Conclusions: Caveolin-1 expression in B16F10 cells promotes cell polarization, directionality, persistent migration and transmigration. This correlates with enhanced turnover of focal adhesions. However, localization of Caveolin-1 to the rear of these metastatic cells was not required.

PARTICIPANTS LIST

Natalia Alonso

nati_alonso05@hotmail.com

Instituto de Biología y Medicina Experimental (IBYME-CONICET), Argentina

Verónica Andreoli

vandreoli@fcq.unc.edu.ar

Universidad Nacional de Córdoba, Argentina

Ana Aranda Iriarte

aaranda@iib.uam.es,

Instituto de Investigaciones Médicas, Madrid, Spain

Francisco Astort

F_astort_lem@hotmail.com

Universidad de Buenos Aires, Argentina

Erika Azorin

erica.azorin@inin.gob.mx

Instituto Nacional de Investigaciones Nucleares, México

LeticiaBaccarini

lbaccarini@qb.fcen.uba.ar

Universidad de Buenos Aires, Argentina

Graciela Boccaccio

gboccaccio@leloir.org.ar

Fundación Instituto Leloir, Argentina

Jose Luis Bocco

jbocco@fcq.unc.edu.ar

Universidad Nacional de Córdoba, Argentina

Juan Jose Bonfiglio

jjbonfiglio@fbmc.fcen.uba.ar

Universidad de Buenos Aires, Argentina

Carlos Bueno

cbueno@qb.fcen.uba.ar

Universidad de Buenos Aires, Argentina

Alan Bush

alerner2@gmail.com

Universidad de Buenos Aires, Argentina

Xose Bustelo

xbustelo@usal.es,

Centro de Investigación del Cancer, Salamanca, Spain

Matías Bustos
mbustos@fcm.uncu.edu.ar
Universidad Nacional de Cuyo, Argentina

Ana Cabanillas
amcaba@fcq.unc.edu.ar
Universidad Nacional de Córdoba, Argentina

Georgina Cardama
gcardama@gmail.com
Universidad Nacional de Quilmas, Argentina

Pamela Carmona
pamelacarmona@uach.cl
Universidad Austral de Chile, Chile

Maria Cecilia Carreras
carreras@ffyb.uba.ar
Universidad de Buenos Aires, Argentina

Rocío Castilla
rocio_castilla@yahoo.com
Universidad de Buenos Aires, Argentina

Lucio Castilla
lucio.castilla@umassmed.edu,
University of Massachusetts Medical School, Worcester, USA

Juan José Chiesa
jjosechiesa@unq.edu.ar
Universidad Nacional de Quilmas, Argentina

John Cidlowski
cidlows1@niehs.nih.gov,
NIEHS, NIH, Research Triangle Park, N.C., USA

Melanie Cobb
melanie.cobb@utsouthwestern.edu,
University of Texas, Southwestern Med. Center, Dallas, USA

Federico Coluccio Leskow
federico@fbmc.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Andrea Comba
andreacomba@hotmail.com
Universidad Nacional de Córdoba, Argentina

Mariana Cooke
mcooke@fmed.uba.ar

Universidad de Buenos Aires, Argentina

Sabrina Copsel

scopsel@yahoo.com.ar

Instituto de Biología y Medicina Experimental (IBYME-CONICET), Argentina

Omar Coso

ocosofbmc.fcen.uba.ar

Universidad de Buenos Aires, Argentina

Gabriela Coux

coux@ibr.gov.ar

Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET/UNR),
Argentina

Piero Crespo

crespop@unican.es,

Universidad de Cantabria, Santander, Spain

Ana Cuenda

acuenda@cnb.csic.es,

Centro Nacional de Biotecnología/CSIC, Madrid, Spain

Alejandro Curino

acurino@criba.edu.ar

INIBIBB-CONICET Argentina

Cora Beatriz Cymeryng

coracymeryng@gmail.com

Universidad de Buenos Aires Argentina

Carlos Davio

cardavio@ffyba.uba.ar

Universidad de Buenos Aires, Argentina

Roger Davis

Roger.Davis@umassmed.edu,

University of Massachusetts Medical School, Worcester, USA

Adriana De Siervi

adesiervi@qb.fcen.uba.ar

Universidad de Buenos Aires, Argentina

Maria Sol Degese

soldgc@gmail.com

Universidad de Buenos Aires, Argentina

Channing Der

cjder@med.unc.edu,

University of North Carolina, Chapel Hill, USA

María Eugenia Díaz
m_euge_diaz@hotmail.com
Universidad de Buenos Aires, Argentina

Alberto Díaz Añel
adiazanel@immf.uncor.edu
Instituto de Investigación Médica Mercedes y Martín Ferreira, Argentina

Maria Lilia Domínguez López
ldmiguez@yahoo.com.mx
Escuela Nacional de Ciencias Biológicas, México

Jozef Dulak
jdulak@mol.uj.edu.pl,
Jagiellonian University, Krakow, Poland

Martín Edreira
mme2@pitt.edu
INGEBI-UBA-CONICET, Ciudad de Buenos Aires, Argentina

M. Eugenia Elguero
carreras@ffyb.uba.ar
Universidad de Buenos Aires, Argentina

Maria Facchinetti
facchinm@criba.edu.ar
INIBIBB-CONICET, Argentina

Mariana Feld
mfeld@fbmc.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Natalia Fernandez
natyfernandez@hotmail.com
Universidad de Buenos Aires Argentina

Mathias Gaestel
gaestel.matthias@mh-hannover.de,
Hannover Medical School, Hannover, Germany

Fiorella Galillo
fgalillo@qb.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Celine Gales
Celine.Gales@inserm.fr,
INSERM, Toulouse, France

Esther Noemi Gerez

cuyro@yahoo.com
CIPYP-CONICET, Argentina

Jimena Giudice
jimena.giudice@gmail.com
Universidad de Buenos Aires, Argentina

Maria Victoria Goddio
vgoddio@qb.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Lorena González
lgonza74@yahoo.com.ar
Universidad de Buenos Aires, Argentina

Carlos Gonzalez Fritz
cbgonzal@uach.cl
Universidad Austral de Chile, Chile

Diego Grinman
dgrinman@qb.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Bernd Groner
groner@em.uni-frankfurt.de,
Georg Speyer Haus Institute, Frankfurt, Germany

Silvio Gutkind
gutkind@dir.nidcr.nih.gov,
NIDCR, NIH, Bethesda, USA

Nancy Hynes
nancy.hynes@fmi.ch,
F.M. Inst. for Biomed. Research, Switzerland

Marcelo Kazanietz
marcelo@spirit.gcrc.upenn.edu,
University of Pennsylvania, USA

Darío Krapf
krapf@ibr.gov.ar
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET/UNR),
Argentina

Andreia Leopoldino
andreiaml@usp.br
Universidade de São Paulo, Brazil

Ruth Lezama Palacios
ralezama@hotmail.com

Escuela Nacional de Ciencias Biológicas, México

Pablo Lorenzano Menna

plmenna@unq.edu.ar

Universidad Nacional de Quilmas, Argentina

Guadalupe Lorenzatti

glorenzatti@fcq.unc.edu.ar

Universidad Nacional de Córdoba, Argentina

Patricia Marini

marini@ibr.gov.ar

Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET/UNR),
Argentina

Carolina Soledad Martínez

carolinasmartinez@gmail.com

Universidad de Buenos Aires, Argentina

Sara Martorelli da Silveira

sara_martorelli@yahoo.com.br

Antonio Prudente Foundation - AC Camargo Hospital, Brazil

Javier Enrique Mendizábal

jemendizabal@yahoo.com.ar

Universidad de Buenos Aires, Argentina

María Elisa Mercau

mercaum@gmail.com

Universidad de Buenos Aires, Argentina

Silvina Beatriz Meroni

smeroni@cedie.org.ar

Centro de Investigaciones Endocrinológicas (CEDIE/CONICET), Argentina

Enrique A. Mesri

EMesri@med.miami.edu

University of Miami, Miami, USA

Carolina Mondillo

caromondillo@hotmail.com

Instituto de Biología y Medicina Experimental (IByME-CONICET), Argentina

Silvia Moreno De Colonna

smoreno@qb.fcen.uba.ar

Universidad de Buenos Aires, Argentina

Nair Hideko Muto

nair.muto@gmail.com

Hospital AC Camargo, Brazil

Julian Naipauer
juliannaipauer@gmail.com
Universidad de Buenos Aires, Argentina

Angel Nebreda
angel.nebreda@irbbarcelona.org
IRB-Barcelona, Spain

Virginia Novaro
vnovaro@gmail.com
Instituto de Biología y Medicina Experimental (IBYME-CONICET), Argentina

Andres Orqueda
aorqueda@qb.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Juana María Pasquini
jpasquin@qb.ffyb.uba.ar
Universidad de Buenos Aires, Argentina

Constanza Pautasso
cpautasso@qb.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Adalí Pecci
apecchi@qb.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Maria Julia Pérez
mariajuliaperezz@yahoo.com.ar
Universidad de Buenos Aires, Argentina

Juan Pablo Petiti
jpetiti@cmefcm.uncor.edu
Universidad Nacional de Córdoba, Argentina

Ernesto Podestá
ernestopodesta@yahoo.com.ar
Universidad de Buenos Aires, Argentina

Diego Presman
diegopres@qb.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Ana Raimondi
rosar71@yahoo.com
Institute of Oncology Angel H. Roffo, UBA, Argentina

Sol Recouvreux
natalia.rubinstein@gmail.com
Universidad de Buenos Aires, Argentina

María Fernanda Riera
friera@cedie.org.ar
Centro de Investigaciones Endocrinológicas (CEDIE/CONICET), Argentina

Martín Rinaldi Tosi
rinaldi@mendoza-conicet.gob.ar
Instituto de Medicina y Biología Experimental de Cuyo, Argentina

Evelyn Robles
evelux07@hotmail.com
Cinvestav, México

Luciana Rocha Viegas
chocha_76@yahoo.com
Universidad de Buenos Aires, Argentina

Ramiro Gonzalo Rodríguez Limardo
ramiro.grl@gmail.com
Universidad de Buenos Aires, Argentina

José Maria Rojas
jmrojas@isciii.es
Instituto Carlos III, Madrid, Spain

Silvia Rossi
srossi@qb.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Natalia Rubinstein
nrubinstein@fbmc.fcen.uba.ar
Universidad de Buenos Aires, Argentina

German Sánchez Alberti
sa.german@hotmail.com
Universidad de Buenos Aires, Argentina

Daiana Sapochnik
dsapochnik@fbmc.fcen.uba.ar
Universidad de Buenos Aires, Argentina

Leonardo Seguin
lseguin@unsl.edu.ar
Universidad Nacional de San Luis, Argentina

Valeria Sigot
vsigot@fbioyf.unr.edu.ar

Centro Binacional (Argentina -Italia) de Criobiología Clínica y Aplicada (CAIC)
dependiente de UNR, Argentina

Ángela Solano
drsolanoangela@gmail.com
Universidad de Buenos Aires, Argentina

Sarah Spiegel
sspiegel@vcu.edu,
Virginia Commonwealth University, Richmond, USA

Laila Suhaiman
suhaiman.laila@fcm.uncu.edu.ar
Universidad Nacional de Cuyo, Argentina

Juan Manuel Teijeiro
teijeiro@ibr.gov.ar
Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET/UNR),
Argentina

Heidi Tissenbaum
heidi.tissenbaum@umassmed.edu,
University of Massachusetts Medical School, Worcester, USA

Johanna Melisa Tocci
jotocci@hotmail.com
Universidad de Buenos Aires, Argentina

Claudia Tomes
ctomes@fcm.uncu.edu.ar
Universidad Nacional de Cuyo, Argentina

Gladys Torres
torresmgladys@yahoo.com
Universidad Nacional Mayor de San Marcos, Perú

Lucas Daniel Trucco
ltrucco@fcg.unc.edu.ar
Universidad Nacional de Córdoba, Argentina

Adrian Turjanski
aturjans@gmail.com
Universidad de Buenos Aires, Argentina

Hery Urra
hery.urra@gmail.com
Universidad de Chile, Chile

Elba Vázquez
elba@qb.fcen.uba.ar

Universidad de Buenos Aires, Argentina

Carolina Villanueva

carolinavillanueva@uach.cl

Universidad Austral de Chile, Chile

Ángeles Vinuesa

angeles_vinuesa@hotmail.com

Universidad de Buenos Aires, Argentina

Pablo Visconti

pvisconti@vasci.umass.edu,

University of Massachusetts, Amherst, USA

Yosef Yarden

Yosef.Yarden@weizmann.ac.il,

Weizmann Institute of Science, Rehovot, Israel