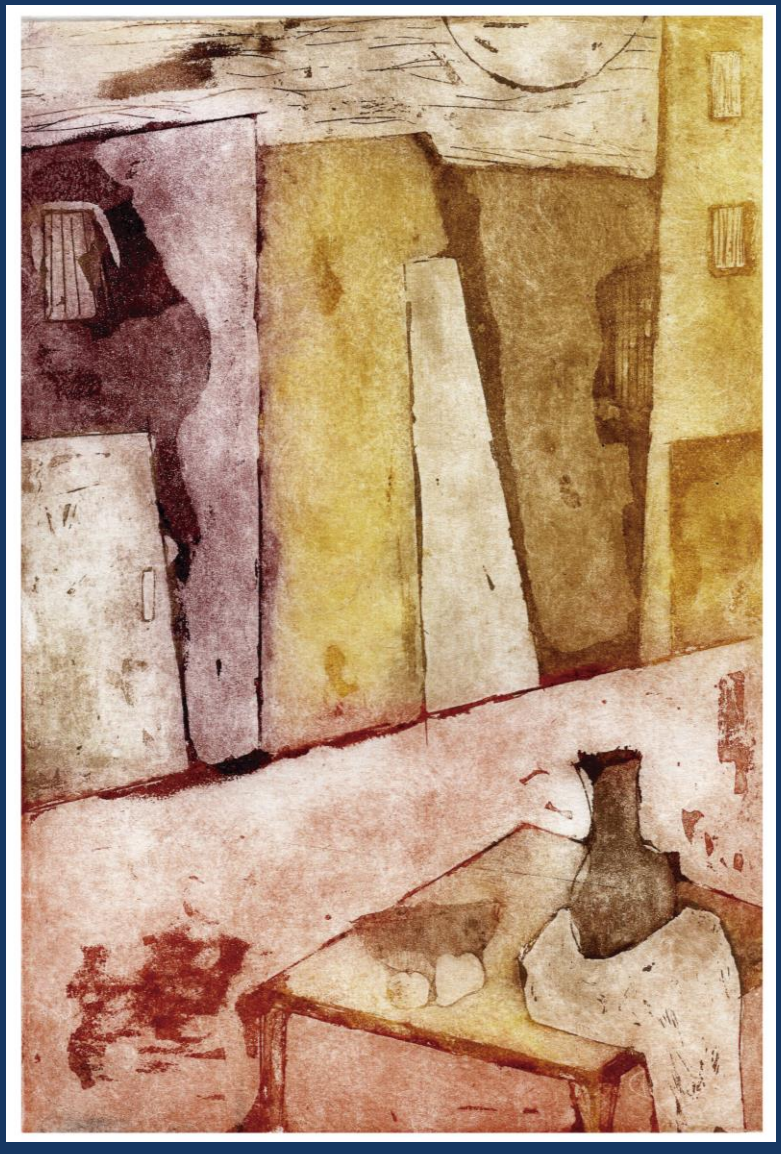
2019

medicina

BUENOS AIRES VOL. 79 Supl. IV - 2019

80° Aniversario



MEDICINA

Volumen 79, Supl. IV, págs. 1-338

medicina

BUENOS AIRES, VOL. 79 Supl. IV - 2019

COMITÉ DE REDACCIÓN

MIEMBROS EMÉRITOS

La Tapa (*Ver* pág. 4)
Atardecer en la tarde
Antonella Ricagni

MEDICINA (Buenos Aires) – Revista bimestral – ISSN 0025-7680 (Impresa) – ISSN 1669-9106 (En línea)

REVISTA BIMESTRAL
Registro de la Propiedad Intelectual N° 02683675
Personería Jurídica N° C-7497
Publicación de la Fundación Revista Medicina (Buenos Aires)
Propietario de la publicación: Fundación Revista Medicina
Queda hecho el depósito que establece la Ley 11723

Publicada con el apoyo del Ministerio de Ciencia, Tecnología e Innovación Productiva.
MEDICINA no tiene propósitos comerciales. El objeto de su creación ha sido propender al adelanto de la medicina argentina.
Los beneficios que pudieran obtenerse serán aplicados exclusivamente a este fin.
Aparece en *MEDLINE (PubMed), ISI-THOMSON REUTERS (Journal Citation Report, Current Contents, Biological Abstracts, Biosis, Life Sciences), CABI (Global Health), ELSEVIER (Scopus, Embase, Excerpta Medica), SciELO, LATINDEX, BVS* (Biblioteca Virtual en Salud), *DOAJ, Google Scholar y Google Books.*
Incluida en el Núcleo Básico de Revistas Científicas Argentinas del CONICET.

Directores Responsables:
Basilio A. Kotsias, Eduardo L. De Vito, Isabel Narvaiz Kantor, Guillermo B. Semeniuk

Secretaría de Redacción: Ethel Di Vita, Instituto de Investigaciones Médicas Alfredo Lanari, Combatientes de Malvinas 3150, 1427 Buenos Aires, Argentina
Tel. 5287-3827 Int. 73919 y 4523-6619
e-mail: revmedbuenosaires@gmail.com – http//: www.medicinabuenosaires.com

Vol. 79, Supl. IV, Noviembre 2019

medicina
BUENOS AIRES, VOL 79 Supl. IV - 2019

REUNIÓN ANUAL DE SOCIEDADES DE BIOCIENCIA 2019

LXIV Reunión Anual de la
Sociedad Argentina de Investigación Clínica (SAIC)

LI Reunión Anual de la
Asociación Argentina de Farmacología Experimental (SAFE)

XXI Reunión Anual de la
Sociedad Argentina de Biología (SAB)

XXXI Reunión Anual de la
Sociedad Argentina de Protozoología (SAP)

IX Reunión Anual de la
Asociación Argentina de Nanomedicinas
(NANOMED-ar)

VI Reunión Científica Regional de la Asociación Argentina
de Ciencia y Tecnología de Animales de Laboratorio
(AACyTAL)

con la participación de
The Histochemical Society

13 - 16 de noviembre de 2019
Hotel 13 de Julio - Mar del Plata

EDITORES RESPONSABLES

Dra. Mónica Costas
Dra. Gabriela Marino
Dr. Pablo Azurmendi

CONTAINING TCAMPKA1 OR TCAMPKA2 IN ENERGY HOMEOSTASIS REGULATION AND CELL CULTURE PROGRESSION IN TRYPANOSOMA CRUZI

Tamara STERNLIEB (1) | Alejandra C. SCHOIJET(2) | Patricio D. GENTA(1) | Guillermo D. ALONSO(2)

INGEBI-CONICET (1); INGEBI- CONICET- UBA (2)

The AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme involved in maintaining energy homeostasis in response to different stresses in many organisms. During the transition between the mammalian host and the insect vector, Trypanosoma cruzi, the causative agent of Chagas disease, faces different types of environmental fluctuations, all of which prompt the parasite to remodel its metabolism. Recently, it was shown that Trypanosoma brucei AMPK is involved in the differentiation from the bloodstream slender to stumpy stage and in surface protein expression changes in response to nutritional stress. This underscores the relevance of AMPK for parasite life cycle progression. We identified four candidate genes for the AMPK subunits of T. cruzi (alpha1 and alpha2 catalytic subunits, beta and gamma regulatory subunits). The beta and gamma subunits are largely conserved in their domain structure relative to the mammalian orthologs. However, the alpha subunits show significant sequence and structure differences from the human counterparts. The presence of these subunits in T. cruzi epimastigotes was confirmed by RT-PCR, Western blot using a phospho-AMPKa specific antibody, mass spectrometry and by kinase activity assays using the specific AMPK substrate SAMS. TcAMPKa1 over-expressing epimastigotes showed a lower growth rate in basal culture conditions compared to the control. On the other hand, alpha2 over-expression had the opposite effect. Additionally, we observed upregulation of AMPK activity under epimastigote starvation, and that dorsomorphin, a specific AMPK inhibitor, also inhibits T. cruzi AMPK. Moreover, each of these subunits could complement S. cerevisiae conditional mutants lacking the respective subunit of the AMPK ortholog SNF1. Finally, starving assays with AMPKa over-expressing parasites also showed a possible role of AMPK in autophagy. Overall, our results show for the first time, the presence of a functional AMPK orthologue in Trypanosoma cruzi.

0784 - MOLECULAR CHARACTERIZATION OF MRE11-RAD50 PROTEINS DURING HOMOLOGOUS RECOMBINATION REPAIR (HRR) IN TOXOPLASMA GONDII

Diego RUIZ (1) | Valeria TUROWSKI(1) | William SULLIVAN(2) | Sergio ANGEL(1)

INSTITUTO TECNOLÓGICO DE CHASCOMÚS (INTECH) (1); PHARMACOLOGY & TOXICOLOGY DEPARTMENT, INDIANA UNIVERSITY SCHOOL OF MEDICINE (2)

The homologous recombination repair (HRR) is critical to genome integrity maintenance during cell replication (late S/G2) but it has not been elucidated in T. gondii. HRR starts with recognition of DNA damage follow by generation of resection ends for which Mre11-RAD50-Nbs1 (MRN) complex is required. In our research group was observed that this parasite´s genome only encodes 50% of HRR proteins described in yeast and mammals suggesting either divergence between these homologues or the presence of parasite-specific components functional in this essential pathway. In fact, T. gondii harbours homologues of Mre11 and RAD50 proteins although no coding sequence to Nbs1 was found. In humans, the complex is composed by a homodimer of Mre11 with endo/exonuclease activity, two RAD50 ATPase and Nbs1. The gene of T. gondii Mre11 (TgMre11) contains an open reading frame encoding a polypeptide with 38.41 % of identity to its human homologue and 535 residues longer. Superimposition between TgMre11 model and HuMre11 crystal structure (RMS 0.84) showed at least three insertions with more than 20 amino acids each on relevant regions for both dimerization and interaction with other proteins or DNA. Then, taking into account that Mre11 and RAD50 are predicted as essential genes in T. gondii, disclosing structural-functional differences between these proteins and their human counterpart as well as characterizing novel T. gondii HRR components might give insights into evolution of this pathway, identify novel drug targets and help to elucidate orthologues in other Apicomplexa.

Supported by NIH 1 R01 AI129807-01 and PICT 2017-2485 grants.

0796 - DEPOLYMERIZATION OF SUMO CHAINS IN T. BRUCEI BLOODSTREAM PARASITES AS A SIGNAL TO CONTROL GROWTH DURING INFECTIONS.

Lucia Ayelen DI MARZIO | Paula Ana IRIBARREN | María Agustina BERAZATEGUI | Vanina Eder ALVAREZ

IIBIO-UNSAM

SUMOylation is a reversible post-translational modification (PTM) that involves the attachment of one SUMO protein or SUMO chains to internal lysines in target proteins. This PTM enables rapid cellular responses, which are essential to pathogenic microorganisms that undergo complex life cycles involving several hosts, as is the case with trypanosomatids. Since we have previously shown that T. brucei is capable of forming SUMO polymeric chains in vitro, our next goals were to determine if this also occurs in vivo and to study their physiological relevance. To achieve this, we generated SUMO chain mutant parasites by replacing the endogenous SUMO alleles with a lysine deficient variant unable to polymerize. This transgenic cell line did not exhibit any evident phenotype and grew equivalent to WT parasites when culture in vitro. However, there were striking differences when using a mouse model of infection. While WT parasite grew uncontrollably killing the host 5-6 days after infection, SUMO mutant parasites limited their growth, generating oscillating parasitemia with prolonged host survival. Knowing that naturally occurring parasites achieve long-term infections inducing differentiation to a quiescent stage, we next examined differentiation kinetics of these parasites by an in vitro approach exposing them to cis-aconitate (CA) at low temperatures. Differentiation from BF to procyclic form (PF) was evaluated by immunofluorescence visualizing the switching from VSG to procyclin. SUMO mutant parasite showed accelerated differentiation kinetics, suggesting that the absence of SUMO chains favors differentiation of the parasite and allows it to successfully establish and maintain an infection.

0805 - CHARACTERIZATION OF THE TCDOT1A AND TCDOT1B ISOFORMS: IMPLICATION OF H3K76 DIFFERENTIAL METHYLATION DURING TRYPANOSOMA CRUZI LIFE CYCLE.

Malena BALESTRASSE | Milena MASSIMINO STEPÑICKA | Guillermo Daniel ALONSO | Josefina OCAMPO

INGEBI-CONICET

Trypanosoma cruzi, the etiologic agent of Chagas Disease, affects a large number of the population in Latin America. It has a complex life cycle alternating between a mammalian host and the vector insect, Triatoma infestans. This cycle consists of three well-defined stages: amastigotes, epimastigotes and trypomastigotes. As the parasite faces different environments, it requires changes in gene expression in order to survive. Hence, gene expression regulation might be a key aspect to understand adaptation. Despite Trypanosomes gene expression is mainly regulated post transcriptionally, there are evidences that chromatin state influence it. Recent studies have shown that DOT1 methyltransferases homologues, called DOT1a and DOT1b, are involved in the methylation of lysine 76 of histone H3 in T. cruzi. In T. brucei, DOT1a mediates H3K76 mono and di-methylation, whereas DOT1b catalyzes H3K76 tri-methylation. However, these two enzymes remain poorly characterized. In this project, we investigated the relevance of TcDOT1a and TcDOT1b during cell

cycle and the metacyclogenesis process. Therefore, to evaluate the catalytic activity using heterologous complementation, we have successfully cloned and transformed a null DOT1 yeast strain with TcDOT1a. Additionally, to analyze the isoforms subcelular location and their effects on cell cycle progression and differentiation, we have cloned the TcDOT1 isoforms in a pRibotex vector with an N-terminal HA-tag and transfected epimastigotes of CL Brener strain. Nevertheless, the overexpression was toxic for the cell. Therefore, we decided to switch to an inducible vector. Overall, our data will be useful to further understand the role of the DOT1 isoforms and the differential methylation of H3K76 in T. cruzi. In the long term, our findings might unravel new targets for antiparasitic drugs.

0808 - CHARACTERIZATION OF HEPATITIS C VIRUS INFECTIONS IN MAR DEL PLATA AND ANALYSIS OF POTENTIAL DIRECT ANTIVIRAL RESISTANCE

Bruno SALDAIN (1) | Lorena SANTANA(2) | Luciana BARBINI(1)

DPTO. DE QUIMICA Y BIOQUIMICA, FACULTAD DE CS EXACTAS Y NATURALES, UNMDP - CONICET (1); SERVICIO DE LABORATORIO, HIGA OSCAR ALENDE, MAR DEL PLATA (2)

About 71 million people are chronically infected with hepatitis C virus (HCV), at risk to evolve to cirrhosis and hepatocellular carcinoma. HCV is distributed in genotypes (gts), associated with the natural history of infection and antiviral response. Direct-acting antivirals (DAAs) are the current drugs used for the treatment of chronic infections, curing 95 % of them. Daclatasvir inhibits the multifunctional NS5A protein, essential component of the replication complex. The aim of this study was to extend the characterization of HCV infections in Mar del Plata and determine the presence of mutations associated to daclatasvir resistance. Treatment-naïve chronically HCV infected patients, were included, and their clinical information was analyzed. The HCV NS5A gene was amplified by RT-nested PCR from serum samples and sequenced. Genotypes were determined by phylogenetic analysis and the presence of mutations was determined. The characteristics of the patients in this cohort were: mean age 55.25 years; 71.43% males; 42.86 % coinfected with HIV and HCV viral load mean 6.61 log. The phylogenetic analysis showed that most samples (85.71 %) were subgt 1b and 14.29 % was 1a, in agreement with the reported most prevalent subgts in the region. All the samples showed mutations in the NS5A gene nucleotide sequences. The analysis of NS5A proteins showed that all samples presented aminoacidic substitutions, but most of them were not associated to resistance. Only a few samples presented mutations that can be associated to antiviral resistance (Y93D and P58R), because they are localized at the binding site of the antiviral. The presence of other aminoacids at this site was reported to reduce daclatasvir activity. In conclusion, new HCV infections in Mar del Plata are produced by the already circulating most prevalent subgts in the region. Actually, local HCV does not exhibit significant mutations related to resistance to one of the most used DAAs.

0827 - FUNCTIONAL CHARACTERIZATION OF BABESIA BOVIS PLP1 THROUGH HEMOLYSIS ASSAYS AND GENERATION OF A KNOCK OUT STRAIN

Martina Soledad PAOLETTA (1) | Ludmila LÓPEZ ARIAS(2) | José Manuel JARAMILLO ORTIZ(1) | Paul Alan LACY(3) | Jacob Michael LAUGHERY(3) | Carlos Esteban SUAREZ(3) | Marisa Diana FARBER(1) | Silvina E. WILKOWSKY(1)

INSTITUTO DE AGROBIOTECNOLOGÍA Y BIOLOGÍA MOLECULAR, INTA-CONICET (1); UNIVERSIDAD NACIONAL DE HURLINGHAM (2); UNITED STATES DEPARTMENT OF AGRICULTURE - WASHINGTON STATE UNIVERSITY (3)

Bovine babesiosis is a tick-borne disease caused by parasites of the Babesia genus affecting livestock production worldwide. Proteins implicated in life cycle progression of parasites are key factors in host-pathogen interaction. We have previously identified in B. bovis a family of Perforin-Like Proteins (PLP) involved in pore formation and erythrocyte damage. One member, PLP1, is expressed and exposed to the host immune system during infection. The aim of this study was to determine the function of PLP1 and its contribution to parasite's pathogenesis. The recombinant MACPF domain (responsible for pore formation in PLPs) of PLP1 was expressed and hemolysis assays were done incubating this protein with bovine RBCs. Cell lysis was expressed as a percentage of maximum hemoglobin release with Triton X-100 treatment. High hemolysis levels (> 80 %) were obtained at [rMACPF] >80 nM, and pH >5. The hemolysis activity was not affected by changes in [Ca^{2+}]. A B. bovis knock out (KO) strain was generated by disruption of plp1. Parasites were transfected with a plasmid to guide replacement of plp1 with an egfp-bsd fusion gene. Integration and disruption of the plp1 gene was confirmed by PCR and Southern blot analysis. The KO phenotype was evaluated by observation of in vitro replication in bovine RBC cultures. KO parasites showed normal rates of replication and development. Yet, an unusual phenotype of multiple parasites accumulated within a single RBC was observed, suggesting a possible defect in egress. This phenotype was already reported for T. gondii plp1 KO. Current studies are aimed to compare transcription levels of other members of the plp family between KO and WT strains. Future studies of in vivo replication of the KO strain in experimental bovine and tick infections will help to determine if plp1 plays a role on another stage of parasite's life cycle.

0832 - OVER-EXPRESSION OF TBRRM1 LEADS TO ABERRANT PHENOTYPES AND CELL DEATH IN TRYPANOSOMA BRUCEI PROCYCLIC CELLS

Analía Gabriela NÍTTOLO | Daniel Oscar SÁNCHEZ | Gabriela LEVY

IIBIO-UNSAM

Since transcription in trypanosomatids is polycistronic, regulation of gene expression occurs mainly at the post-transcriptional level by RNA binding proteins. In our lab we study the gene expression regulation in T. brucei, particularly we focus on elucidating the function of the RNA binding protein, TbRRM1. TbRRM1 has three RRM domains in the amino-terminal region followed by two zinc finger domains and a region rich in dipeptides (Asp/Glu)-Arg which precedes the RS domain characteristic of the SR protein family. Previously, we have demonstrated that TbRRM1 is essential for survival in procyclic and bloodstream form stages of T. brucei since its silencing by RNAi affects the growth curve, produces aberrant phenotypes and promotes cell death by a mechanism compatible with apoptosis. The aim of the present work was to contribute to the elucidation of TbRRM1 function through the study of the effects of its over-expression. For that purpose, we have established a system for the inducible over-expression of a FLAG-tagged TbRRM1 and different mutants. Results showed that over-expression of 3xFLAG-TbRRM1 was deleterious for parasite survival. Surprisingly, the parasites displayed an aberrant morphology that was previously observed when TbRRM1 was depleted, suggesting that both silencing and over-expression of TbRRM1 produce a similar phenotype. In addition, since both silencing and over-expression of TbRRM1 are lethal for parasites, it could be concluded that TbRRM1 is a relevant protein whose levels must be finely regulated.

0842 - MONOSISTRONIC EXPRESSION SYSTEM AND ENDOGENOUS GENE LABELING THROUGH THE T2A STRATEGY IN TRYPANOSOMA CRUZI

Giannina CARLEVARO | Gabriela NIEMIROWICZ | León BOUVIER | Juan MUCCI