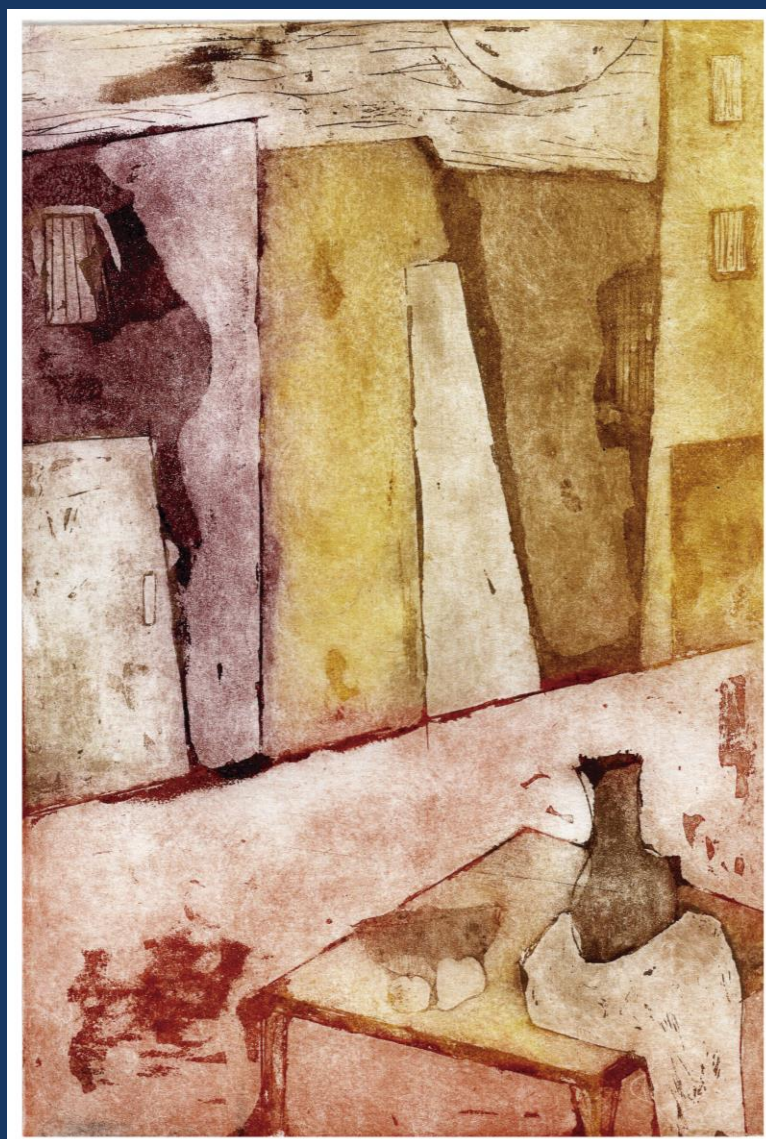


2019

medicina

BUENOS AIRES VOL. 79 Supl. IV - 2019

80° Aniversario



MEDICINA

Volumen 79, Supl. IV, págs. 1-338

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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.

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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.

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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

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bromodomain inhibitors. We determined the dissociation constant for bromosporine and I-BET151.

0653 - EFFECT OF THE CALCIUM BINDING PROTEIN TCCAL1 ON THE PROLIFERATION OF TRYPANOSOMA CRUZI

Jessica RODRÍGUEZ | Karina GÓMEZ | Mariana POTENZA
IMMUNOLOGY OF TRYPANOSOMATIDS INFECTION LAB. INGEBI-CONICET

Calcium homeostasis is implicated in essential processes of *T. cruzi* life cycle. Although several calcium binding proteins have been identified in *T. cruzi* genome, their role in Ca²⁺ signaling remain still unknown. TcCAL1 is a hypothetical protein identified through proteome analysis of *T. cruzi*, which has two EF-Hand domains involved in Ca²⁺ binding. The hypothesis to be tested in this study was that TcCAL1 overexpression could trigger alterations in the intracellular calcium homeostasis leading an effect on epimastigote proliferation. For this purpose, the gene encoding *tccal1* was amplified by PCR and cloned fused to a 6x histidine tag into the pTRES expression vector. The recombinant plasmid pTRES/*tccal1*x6His was used to transfect epimastigotes from *T. cruzi* Y or CL Brener strains. Selection of transgenic cultures was carried out growing parasites in presence of increasing concentrations of Geneticin. Overexpression of TcCAL1x6His fusion protein was confirmed through western blot and affinity chromatography. Growth curves of recombinant cultures from Y or CL Brener strains were performed in triplicate, counting the parasites in a Neubauer chamber. Parasite cultures carrying the empty vector pTRES were used as controls. Data obtained were processed using GraphPad Prism 6.0 Software and subjected to statistical analysis by two-way ANOVA test. The preliminary results showed that the overexpression of TcCAL1x6His affects the growth curve of CL Brener strain at the stationary phase but the proliferation Y strain is not affected. Additional experiments are being carrying out to validate these results and to study the effect of TcCAL1 overexpression on *T. cruzi* differentiation. Future assays including parasites carrying the *tccal1* gene silenced will contribute to validate our hypothesis and to reveal the function of TcCAL1 in calcium homeostasis of *T. cruzi*.

0673 - IDENTIFICATION AND QUANTIFICATION OF PROTEASES IN GERMINAL CELLS OF ECHINOCOCCUS GRANULOSUS

Clara María ALBANI(1) | Joerg REINDERS(2) | Julia FABBRI (1) | Patricia Eugenia PENSEL(1) | María Celina ELISSONDO(1)

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The cestode *Echinococcus granulosus* (Eg) is the causative agent of cystic echinococcosis, a severe neglected zoonosis which has important medical and economic impact. The inner layer of the hydatid cyst or “germinative layer” contains a group of cells called germinative cells (GC). These cells are responsible for cell proliferation and differentiation. Eg has the capability to survive for long periods of time in many mammalian host species. The prolonged survival indicates that the parasite is able to evade host attack for example through the production and release of proteases to digest host proteins. The aim of the present work was to identify and to quantify proteases in Eg GC grown in different conditions using a proteomic strategy. Eg primary cell culture was obtained from hydatid cysts and maintained with weekly splitting during 1-4 month. Cells were culture in normal media and conditioned media (resembling host environment) for different time periods. Then, 50 µl of cells were homogenized in lysis buffer and the peptide content was estimated. Aliquots of 100 µg protein per sample were used for filter-aided sample preparation and the resulting peptides were subjected to nano-LC-MS/MS-analysis. A combined library was set-

up by combining the different runs using the Protein Pilot-software and Uniprot database. We identified a total of 455 proteins in all the studied conditions. We identified and quantified 7 different proteases which were differentially represented in the studied conditions. For example, Calpain A (Accession U6J063) and Mitochondrial processing peptidase beta subunit (Accession U6JE67) were found overrepresented in cells cultured in normal media with a fold change 12 and 2.8 respectively (p<0.05). Through the methodology used, it was possible to describe the presence of several proteases in the GC of Eg, suggesting that some evasion mechanisms are present.

0684 - EXPRESSION PROFILING OF MIRNAS THROUGH METACESTODE DEVELOPMENT IN ECHINOCOCCUS MULTILOCULARIS

Natalia MACCHIAROLI (1) | Matias PEREZ(1) | Marcela CUCHER(1) | Laura KAMENETZKY(1) | Klaus BREHM(2) | Mara ROSENZVIT(1)

IMPAM (UBA-CONICET) (1); UNIVERSITY OF WÜRZBURG, INSTITUTE FOR HYGIENE AND MICROBIOLOGY (2)

The tapeworm (cestode) *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis, a neglected zoonotic disease. MicroRNAs (miRNAs), a class of small non-coding RNAs, are principle regulators of gene expression at the post-transcriptional level and are involved in many different biological processes. In previous work, we described the miRNA repertoire of *E. multilocularis* in vivo metacestode, the stage of sanitary relevance. In this work we described for the first known time the expression profile of the miRNA repertoire through metacestode development in *E. multilocularis*. Small RNA libraries from *E. multilocularis* in vivo metacestodes, in vitro metacestode vesicles and different stages of primary cells were sequenced. Then, miRNA prediction and differential expression analysis were performed. We found a high expression of a few miRNAs, such as miR-71 and miR-9, in all sequenced samples of *E. multilocularis*. The high expression of these miRNAs was conserved in other cestodes, suggesting that these miRNAs may play essential roles in development and survival. Differential expression analysis showed highly regulated miRNAs through metacestode development, suggesting a role in the regulation of developmental timing and/or host-parasite interaction. Some *E. multilocularis* miRNAs are protostome-specific or bilaterian-specific but divergent from host orthologs, and therefore could represent novel biomarkers and/or selective drug targets for echinococcosis infection. The comprehensive identification and expression analysis of *E. multilocularis* miRNAs can help to analyze miRNA function and identify miRNAs potentially useful for the control of alveolar echinococcosis.

0687 - DECIPHERING THE ROLE OF MICRORNAS IN TAPEWORM BIOLOGY: MIR-71 KNOCKDOWN INHIBITS ECHINOCOCCUS MULTILOCULARIS EARLY DEVELOPMENT IN VITRO.

Matías PEREZ (1) | Markus SPILIOTIS(2) | Natalia REGO(3) | Natalia MACCHIAROLI(1) | Laura KAMENETZKY(1) | Nancy HOLROYD(4) | Marcela CUCHER(1) | Klaus BREHM(2) | Mara ROSENZVIT(1)

IMPAM (UBA-CONICET) (1); UNIVERSITY OF WÜRZBURG, INSTITUTE FOR HYGIENE AND MICROBIOLOGY (2); INSTITUT PASTEUR DE MONTEVIDEO, UNIDAD DE BIOINFORMÁTICA, MONTEVIDEO, URUGUAY (3); WELLCOME TRUST SANGER INSTITUTE (4)

Echinococcosis represents a major public health problem worldwide and is considered a neglected disease by the World Health Organization. The etiological agents are *Echinococcus* tapeworms, which display particular developmental traits that imply a complex control of gene expression. MicroRNAs (miRNAs), a class of small regulatory RNAs, are involved in the regulation of many biological processes such as development and metabolism. They act through the repression of messenger RNAs (mRNAs) by