Karina Belén SABALETTE | Vanina CAMPO | Javier DE GAUDENZI

INSTITUTO DE INVESTIGACIONES BIOTECNOLÓGICAS IIBIO-CONICET, UNSAM

Abstract/Resumen: RNA regulons, ribonucleoprotein complexes composed by functionally related mRNAs together with RNA-binding proteins (RBPs), play an important role in the post-transcriptional regulation of gene expression in trypanosomes. The RNA-binding protein U-rich RBP 1 (TcUBP1) targets numerous mRNAs encoding cell-surface glycoproteins preferentially expressed in infective trypomastigotes of Trypanosoma cruzi, the agent of Chagas disease. All these mRNA targets have common 3'-UTRs with a 50-nt linear sequence motif proximately upstream of the signature RNA element for TcUBP1. Overexpression of TcUBP1-GFP in replicative epimastigotes resulted in changes in the subcellular localization of these transcripts from the posterior region to the perinuclear region of the cell, as is typically observed in infective trypomastigotes. We hypothesize that mRNA localization is a mechanism for stage-specific gene regulation in trypanosomes. To test this possibility, we used the wild-type T. cruzi CL-Brener strain and performed a trypomastigote-to-epimastigote differentiation in vitro, incubating the parasites in BHT media supplemented with BFS 10 %. During this differentiation process, the expression and localization of cell-surface associated glycoprotein transcripts were followed by RNA FISH, with a specific Cy3-oligo probe, at different time-points ranging from 1 to 42 days. After incubation and washing, the RNA signal changed from being uniformly distributed in the cytosol (in the trypomastigote form, day 1) to be preferentially restricted to the posterior region of the cell (in the epimastigote form, day 42). Indirect immunofluorescence labeling of cells with an anti-TcCruzipain polyclonal serum detected these mRNAs in a subcellular region that matches to reservosomes, suggesting that RNA localization mechanisms triggered by TcUBP1 might be involved in the regulation of stage-specific protein expression.

0867 - GENOTYPING FASCIOLA HEPATICA BY ITS1 AND RAPDS SUGGESTS DISTINCTIVE SOUTH AMERICAN GENETIC DIVERSITY AND HOST AFFINITY

Silvana SCARCELLA (1) | Florencia BIANCO(1) | Roberto MERA Y SIERRA(2) | Gisela NEIRA(2) | Maria Victoria SOLANA(1) | Hugo SOLANA(1) | Estefan MIRANDA MIRANDA(3)

CIVETAN CONICET (1); CIPAR (2); INIFAP- INSTITUTO NACIONAL DE INVESTIGACIONES FORESTALES AGRICOLAS Y PECUARIAS (3)

Abstract/Resumen: The common liver fluke (Fasciola hepatica) is a major cause of economic losses to agriculture all over the world, with cost estimated at US$ 2,000 million per annum, Given that the existence of genetically different populations of F. hepatica could allow, against any selection pressure, natural or artificial (for use fasciolicides products and/or control measures), one or more populations of F. hepatica to be able to survive and create resistance or adaptability to such selective pressure. It's important to characterize the different isolation of the liver fluke. The aim of the present work was to characterize genetically adult F. hepatica isolates from cattle, pigs, buffaloes and donkeys from different regions of South American, using sequence analysis of ribosomal ITS1 and RAPD-PCR. Genotyping of Fasciola hepatica DNA samples derived from, cattle, pig, buffalo, and donkey collected from different regions of South America, were performed using the F. hepatica Internal Transcribed Spacer (ITS) sequencing, as well as RAPDs-PCR. Phylogeny assessment derived from multiple sequence alignment (MSA) of ITS sequences, exhibit a distinctive South American geographical pattern compared against F. hepatica reported ITS sequences from around the world, MSA analysis of ITS sequences also showed the F. hepatica ITS haplotypes found in south America are consistent with other reported ITS haplotypes. Further phylogenetic assessment of the electrophoresis band pattern of RAPDs-PCR amplicons, suggest the parasite's genome contains markers that may reveal a host preference. Further assessment revealed two major F. hepatica groups within the South American isolates that clearly diverged from each other, one containing parasites obtained from swine and donkeys and other found in bovid with two additional branch subdivisions of the latter group one containing water-buffalos and a second containing only cattle.

0869 - MOLECULAR CHARACTERIZATION OF CIRCULATING TREPONEMA PALLIDUM CLUSTERS IN PEDIATRIC PATIENT

Luciana Noemi GARCIA (1) | Nicolas MORANDO(2) | Adrian OTERO(1) | Samanta MORONI(1) | Guillermo MOSCATELLI(1) | Nicolas GONZALEZ(1) | Analia TOLEDANO(3) | Ana CARATOZZOLO(3) | María Ángeles PANDO(4) | Jaime ALTCHEH(1)

SERVICIO DE PARASITOLOGÍA Y CHAGAS - HOSPITAL DE NIÑOS "RICARDO GUTIÉRREZ" (1); INBIRS, CONICET, FAC. MEDICINA-UBA (2); SERVICIO DE INFECTOLOGÍA, HOSPITAL DE NIÑOS RICARDO GUTIERREZ (3); INSTITUTO DE INVESTIGACIONES BIOMÉDICAS EN RETROVIRUS Y SIDA (CONICET), FACULTAD DE MEDICINA, UBA (4)

Abstract/Resumen: Syphilis is a public health problem with a sustained increase being the incidence of congenital syphilis (Sc) of 1.7 ‰ live births. Currently there are no techniques with sufficient sensitivity and specificity for the diagnosis of Sc Our group recently started with maternities a multicenter study to evaluate the use of molecular biology techniques (MBT) such as PCR, for the diagnosis of Sc. In Argentina there aren't data about MBT in the diagnosis of syphilis or about the strains of Treponema pallidum pallidum (Tpp) in the pediatric population. Our objective was to evaluate the use of PCR in the diagnostic of syphilis in children and examine the clusters of Tpp by DNA sequencing. Pilot study in 5 pediatric cases of syphilis. Different lesion swabs (total= 8 samples) were processed for DNA extraction (QIAamp DNA Blood Mini Kit) followed by PCR for the genes of Tpp: Tp47kDA (conventional PCR) and dnaA using Taqman® probes (real time PCR - qPCR). Additionally, nested PCR for the TP0136 and TP0548 genes were performed in the samples and the sequences were subsequently purified and sequenced by commercial kit (BigDye™) in a genetic analyzer (3500 analyzer).Then, edition and alignment analysis were performed compared to the reference sequences of cluster SS14 (GenBank CP004011.1) and Nichols (GenBank CP004010.2). The different MBT (PCR, nested PCR and qPCR) concordance in an 88 % while 5 samples (at least one swab per patient) from different regions (soft palate, perianal, palms) were positive, 2 samples (perianal, tongue) were negative. Only 1 sample (perianal) was only positive by qPCR. In the sequencing analysis the predominant Tpp clade was Nichols (n= 4) over SS14 (n= 1). The results of PCR determination in blood of these patients is still in process. This study is the first one conducted in the pediatric population in our country for the detection of syphilis by BMT. As recently studies in Argentina reports, the Nichols clade in our study was greater as 10%, although in our pediatric population the prevalentce was higher (80 %) that in adults (26,8 %). The application of BMT in the detection of Sc is a promising alternative; the development of a large-scale and multicentric study will asses the effectiveness of the BMT and provide valuable information on local transmission networks of Tpp.

0870 - TRYPANOSOMA CRUZI EXPERIMENTAL INFECTION IN MICE DEFICIENT FOR MITOCHONDRIAL CYCLOPHILIN D

Natalia MILDUBERGER (1) | Carolina GONZALEZ(2) | Patricia BUSTOS(1) | Alina Elizabet PERRONE(1) | Miriam POSTAN(3) | Jacqueline BUA(4)