Trypanosoma cruzi coding transcriptome in response to gamma radiation

Pereira MA[1], Imada EL[1], Grynberg P[2], Vieira HGS[3], Kaczorowski D[3], Macedo AM[1], Machado CR[1], Franco GR[1]

[1]Departamento de Bioquímica e Imunologia, UFMG, Belo Horizonte, Brasil; [2]Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brasil; [3]Garvan Institute of Medical Research, Sydney, Australia

Trypanosoma cruzi, the etiologic agent of Chagas disease, is a kinetoplastid organism highly resistant to DNA damage caused by ionizing radiation. After a dose of 500 Gy of gamma rays, the genomic DNA is fragmented. Interestingly, the parasite is able to restore the chromosomal bands pattern in less than 48 hours. Previous studies using microarrays and 2D PAGE followed by MS/MS analyzed how gamma rays affect *T. cruzi* gene expression. Microarray analysis showed that transcripts related to basal metabolic functions were down-regulated. In contrast, the up-regulated category was mainly composed by obsolete sequences, hypothetical proteins and Retrotransposon Hot Spot genes. Proteomic analysis indicated that active translation is essential for the parasites recovery from ionizing radiation damage. The presence of shorter protein isoforms after irradiation suggests the occurrence of post-translational modifications and/or processing in response to gamma radiation stress. Our study aims to analyze the gamma radiation effect on the *T. cruzi* transcriptional profile by high-throughput RNA sequencing (RNA-Seq) and to increase our knowledge on the molecular mechanisms related to the parasite resistance do ionizing radiation. Epimastigote cells from CL Brener strain were exposed to a dose of 500 Gy in a cobalt (60Co) irradiator. Total RNA was extracted from non-irradiated (control sample) and irradiated cells (4, 24 and 96 hours post-irradiation). Two biological replicates were produced for each condition. RNA-seq paired-end strand specific libraries were prepared using poly(A) enrichment/dUTP incorporation and sequenced on the Illumina HiSeq 2500 platform. Approximately 210 millions paired-end reads were obtained. FastQC was used for quality control. Reads were edited *in silico* to remove ERCC92 sequences, adapters and low quality regions. In order to generate a reference transcriptome all samples including biological replicates were combined into a single RNA-Seq data set and assembled by Trinity with different k-mers (25, 27, 29 and 31-mers). Trinity 31-mers assembly was selected to downstream analysis after evaluation by Transrate. A total of 75,798 transcripts (44,773 genes) were generated with an average contig length of 717.52 bp and N50 value equal to 1584 bp. Next steps include transcripts redundancy decrease, transcriptome annotation, differential expression and functional analysis. This study will help to understand how the parasite can handle such a harmful stress.

Financial Support: Capes, CNPq, FAPEMIG, Garvan Institute