Tabela 1: Principais miRNAs diferencialmente expressos em linfócitos T CD4+

HTLV-1 study in Hemotherapy Center of Ribeirão Preto

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INTRODUCTION

Since 1991, the Hemotherapy Center of Ribeirão Preto has been concerned with the studies regarding the human retroviruses among healthy blood donors and patients from the Clinical Hospital. Molecular diagnostic techniques for blood-transmitted viruses like HIV-1 and HTLV-1/2 have been developed and improved in our lab. Moreover, there are several researches investigating the immune and molecular mechanisms involved in the transmission and development of HTLV-symptomatic disease.

OBJECTIVES

In 2009, our group decided to develop a molecular platform using the real time PCR for confirmatory and discriminatory diagnosis of HTLV-1/2. Suitable viral genomic region was chosen for the primers and probes and internal amplification controls (IC) was developed. Finally, all assay validation processes were carried out. This platform has been developed in partnership with Gene ID S/A company working with DNA analysis. The objective is to develop of diagnostic commercial kit HTLV-1/2 diagnosis.

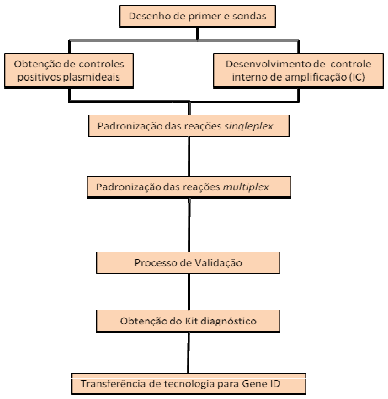

Figure 1: Experimental strategy to develop a HTLV molecular platform

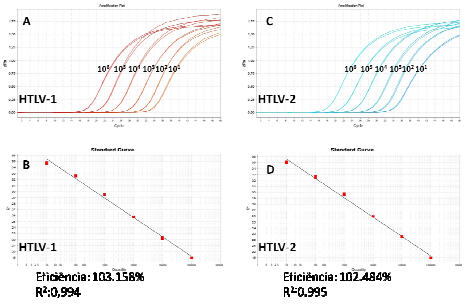

Figure 2: Amplification plots and standard curves of the TaqMan® real-time PCR in singleplex format. The amplification plots and standard curves were obtained based on 10-fold dilutions (10^6 to 10^1 copies/reaction) of plasmid positive controls for HTLV-1 (CP01) and HTLV-2 (CP02). (A) and (C) show the amplification plot of HTLV-1 and HTLV-2 respectively. (B) and (d) show the HTLV-1 and HTLV-2 standard curves. The linear correlation (R2) and efficiency are suitable.

Besides, microRNAs (miRNAs) functional studies have also been developed because of the fact miRNAs could be involved in HTLV-1 pathogenesis. Differently expressed miRNAs from T CD4+ lymphocytes of HTLV-1 infected asymptomatic and symptomatic individuals were compared. The results of this work will contribute to clarify the viral latency mechanisms in infected cells and the factors promoting the development of disease.

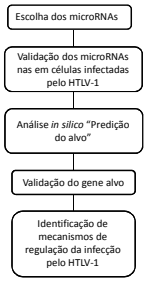

Figure 3: Experimental strategy to microRNAs HTLV studies

miRNAs	*HAC*	*HAM/TSP*	*Localização*	*Mapa Genético*	*Organização*
let-7a	69.46	0.59	intergenic	9q22.32	Cluster
let-7d	1.52	0.38	antisense	9q22.32	Cluster
let-7i	3.14	0.79	intergenic	12q14.1	Single
miR-15b	1.61	5.30	sense	3q25.33	Cluster
miR-23a	0.20	2.01	intergenic	19p13.12	Cluster
miR-26a-1	3.82	1.09	sense	3p22.2	Single
miR-26b	2.73	0.68	sense	2q35	Single
miR-34a	0.43	3.16	intergenic	1p36.22	Single
miR-125b-1	3.09	0.46	intergenic	11q24.1	Single
miR-155	5.12	1.66	sense	21q21.3	Single
miR-221	3.13	0.27	intergenic	Xp11.3	Cluster
miR-223	15.07	1.53	intergenic	Xq12	Single
miR-187	0.41	0.12	intergenic	18q12.2	Single
miR-199a	3.59	0.57	antisense	19p13.2	Single
miR-203	19.30	2.80	intergenic	14q32.33	Single
miR-17-5p	1.92	0.27	sense	13q31.3	Cluster

Table 1: miRNAs from T CD4+ lymphocytes of HTLV-1 differently expressed

In addition, other studies have been performed for evaluation of the immunosuppression mechanisms of human mesenchymal stromal cells (MSC) on HTLV-1 infected lymphocytes and investigation of the HTLV-1 dissemination in infected individual. MSCs have a central role in hematopoiesis and powerful immunomodulation effects that control the immune cells proliferation. Therefore, we are evaluating if MSCs can be infected HTLV-1 and the functional and physiological changes that this retrovirus causes on these cells. Besides that, we are studying the gene networks involved in HTLV-1 gene modulation by MSCs.

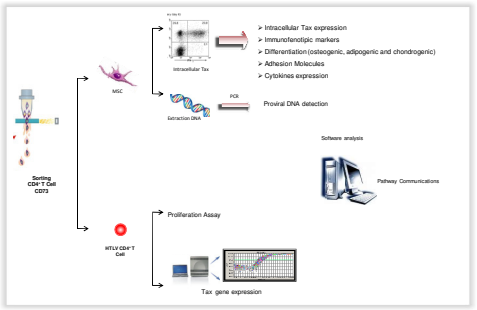

Figure 3: Experimental strategy to evaluation of the immunosuppression mechanisms of human mesenchymal stromal cells (MSC) on HTLV-1 infected lymphocytes

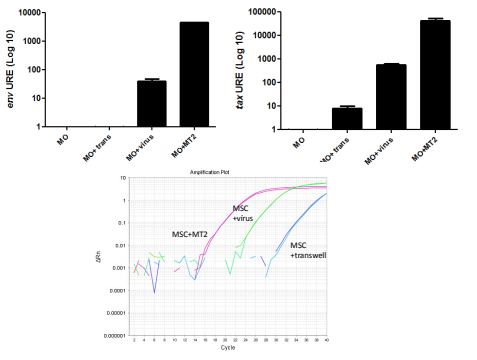

Figure 4: HTLV-1 infect ion in MSCs cells after coculture with MT2 cells

CONCLUSION

These results can help in the treatment of the HTLV-1 infection because will allow the identification of biomarkers and therapeutic targets of the virus. The data of these studies will be useful for understanding of the involvement of the immune system in HTLV-1 infection and will contribute to examine the clinical course of the disease.

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