NEAR NORMALIZATION OF IMMUNE ACTIVATION IN PLWH ON LONG-TERM SUPPRESSIVE ART

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Óscar Brochado Kith[1], Isidoro Martínez González[1], Juan Berenguer[2] , Luz M. Medrano[1], Juan González-García[3], Pilar García-Broncano[4], María Á. Jiménez Sousa[1], Ana Carrero[2], Victor Hontanon[3], M Ángeles Muñoz-Fernandez[2], Juan Carlos López-Bernardo[2], Amanda Férnandez-Rodríguez[1], Salvador Resino[1] for the GeSIDA study group

[1]Institute of Health Carlos III, Madrid, Spain, [2]University Hospital Gregorio Marañon, Madrid, Spain, [3]La Paz University Hospital, Madrid, Spain, [4]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA.

BACKGROUND

In HIV-infected patients, chronic immune activation and inflammation persist after suppressive combination antiretroviral therapy (cART). We compared gene expression and biomarkers in peripheral blood between HIV infected patients on long-term suppressive cART (HIV-group) and age-matched healthy controls (HC-group).

METHODS

Cross-sectional study of 22 subjects in HIV-group with HIV-RNA <50 copies/ml and CD4+ T-cells ≥500 cells/mm for more than one year before sampling, and CD4/CD8 ratio ≥1 at sampling (**Table 1**). RNA-sequencing of poly(A)-RNAs was performed from peripheral blood mononuclear cells (PBMCs). Thirteen T-cell subsets were evaluated by flow cytometry and 32 plasma biomarkers by ELISA multiplex immunoassays. All p-values were corrected by the false discovery rate (q-values).

Characteristics	Healthy controls	HIV-infected patients	p-value
No.	14	22	
Age (years)	50 (46; 53)	51 (48; 53)	0.612
Gender (male %)	7 (50%)	13 (59.1%)	0.593
BMI (kg/m^2)	24.3 (22.7; 27.1)	25.3 (23.5; 27.1)	0.487
Risk group of HIV infection			
Heterosexual	-	10 (45.4%)	-
Homosexual	-	11 (50%)	-
Unknown	-	1 (4.6%)	-
Time of HIV infection (years)	-	15.7 (10.7; 20)	-
Antiretroviral therapy			
PI-based	-	5 (22.7%)	-
2NRTI+II-based	-	2 (9.1%)	-
2NRTI+NNRTI-based	-	13 (59.1%)	-
Others	-	2 (9.1%)	-
Time on cART (years)	-	10.6 (5.3; 16.6)	-
HIV markers			
Prior AIDS diagnosis	-	9 (40.9%)	-
Nadir CD4+ T-cells	-	275.5 (143; 343)	-
Nadir CD4+ ≤200 cells/mm^3	-	9 (40.9%)	-
CD4+ T-cells	-	906 (692; 1045)	-
CD4+ ≥500 cells/mm^3	-	22 (100%)	-
Time with CD4+ ≥500 cells/mm^3 (months)	-	98 (52; 150)	-
CD4/CD8	2.3 (2.1; 3.7)	1.6 (1.2; 2.1)	0.001
CD4/CD8 ≥1	14 (100%)	22 (100%)	0.999
uVL (HIV-RNA <50 copies/ml)	-	22 (100%)	-
Time with uVL (months)	-	76 (35; 108)	-

Table 1. Clinical and epidemiological characteristics of the patients that conform the study.

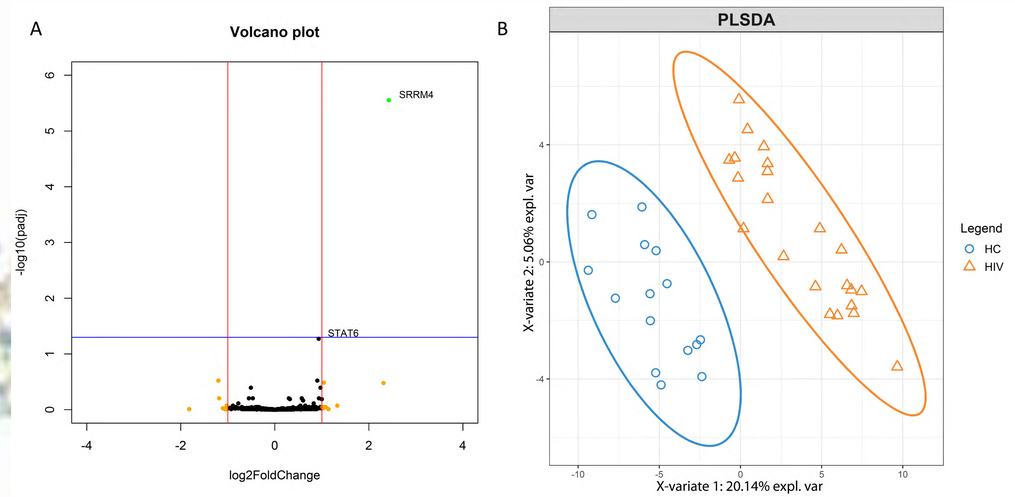

Figure 1. A) Volcano plot representing q-values and Log2Foldchange of genes. B) Representation of groups of samples trough PLS-DA distribution based on gene expression.

- Gene expression and plasma biomarkers showed slight differences between HIV-group and HC-group.
- Only SRRM4, which is involved in alternative RNA splicing events, was differentially expressed.
- Relaxed conditions revealed alterations in ribosome-related pathways.

RESULTS

Only the serine/arginine repetitive matrix 4 (SRRM4) gene was differentially expressed between HIV and HC groups (q-value≤0.05 and fold-change≥2) (**Figure 1A**). However, 147 differentially expressed genes were found with a more relaxed threshold (p-value≤0.05 and fold-change≥1.5), which were used in a partial least squares discriminant analysis (**Figure 1B**). Sixty-seven of them showed variable importance in projection (VIP≥1), which were selected for pathway analysis (**Table 2**). Significant Ribosome-related pathways were represented by six ribosomal genes (RPs): S27 (RPS27), L18a (RPL18A), L8 (RPL8), L26 (RPL26), L4 (RPL4), and S21 (RPS21), all of them downregulated in the HIV-group. T-cells subset and plasma biomarkers were also analyzed, but none of them showed significant differences (q-value>0.05). However, non-corrected p-values showed higher values of CD4 Treg cells (CD4 CD25 CD127), MCP-1, and sVEGF-R1 in the HIV-group (p-value≤0.05). Correlation patterns between RNA-seq expression and peripheral biomarkers (T-cells and plasma) were different between HC and HIV groups.

KEGG	Hits	Gene Symbol	p-value	q-value
hsa03010:Ribosome	6	RPS27A, RPL18A, RPL8, RPL26, RPL4, RPS21	4.43E-05	3.27E-03
Gene Ontology	**Hits**	**Gene Symbol**	**p-value**	**q-value**
GO:0006413~translational initiation	7	RPS27A, RPL18A, EIF1AX, RPL8, RPL26, RPL4, RPS21	2.77E-06	9.33E-04
GO:0006614~SRP-dependent cotranslational protein targeting to membrane	6	RPS27A, RPL18A, RPL8, RPL26, RPL4, RPS21	7.75E-06	1.30E-03
GO:0019083~viral transcription	6	RPS27A, RPL18A, RPL8, RPL26, RPL4, RPS21	1.82E-05	2.04E-03
GO:0000184~nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	6	RPS27A, RPL18A, RPL8, RPL26, RPL4, RPS21	2.44E-05	2.05E-03
GO:0006364~rRNA processing	6	RPS27A, RPL18A, RPL8, RPL26, RPL4, RPS21	3.88E-04	2.58E-02
GO:0006412~translation	6	RPS27A, RPL18A, RPL8, RPL26, RPL4, RPS21	8.29E-04	4.55E-02
GO:0016197~endosomal transport	3	PICALM, CHMP4B, WAS	1.59E-02	5.37E-01
GO:1902004~positive regulation of beta-amyloid formation	2	PICALM, CLU	2.03E-02	5.78E-01
GO:0051131~chaperone-mediated protein complex assembly	2	LONP1, CLU	3.73E-02	7.59E-01
GO:0098609~cell-cell adhesion	4	PICALM, ATXN2L, CHMP4B, TES	4.45E-02	7.84E-01
GO:1901216~positive regulation of neuron death	2	PICALM, CLU	4.85E-02	7.82E-01

Table 2. Summary of significant pathways from KEGG and Gene Ontology databases.

CONCLUSIONS

Immune activation and inflammatory biomarkers were close to normalization in HIV-infected patients on long-term suppressive cART, compared to HC-group. However, residual alterations remained at the gene expression of PBMCs, which still reveal the impact of HIV infection in these patients.