THE MOLECULAR CARGO OF EXOSOMES IS ABLE TO DISCRIMINATE BETWEEN PLWH WITH OR WITHOUT NADCS

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I. Backgound

- Non-Aids Defining Cancers (NADCs) are the main cause of morbidity and death from non-AIDS events in people living with HIV (PLWH), whose death rate is higher than in the general population without HIV infection but who suffer from these same NADCs. However, the mechanisms that explain this are unknown.
- It has been suggested that exosomes derived from HIV infected cells can promote and enhance cancer *in vitro*.
- Herein, we have analyzed the proteomic and miRNA cargo of exosomes from PLWH with active NADC to identify a molecular profile associated to this condition.

II. Methods

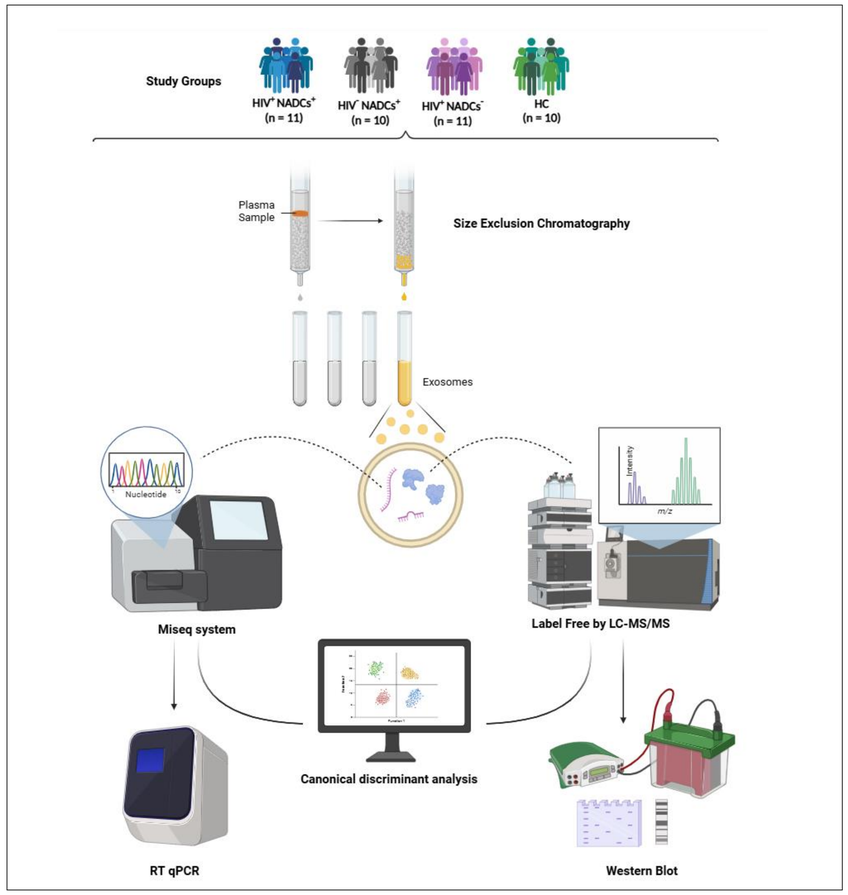


Figure 1. General outline of the methodology: Forty-two participants were included: 11 PLWH on cART (HIV⁺NADCs⁻), 11 on cART with NADC (HIV⁺NADCs⁺), 10 HIV-uninfected with NADCs (HIV⁻NADCs⁺), and 10 healthy volunteers (HC). Size Exclusion Chromatography (SEC) was employed to isolate plasma exosomes. Protein and miRNAs content of exosomes isolated from plasma were analyzed by liquid chromatography coupled with mass spectrometry (LC-MS/MS) (proteins) and by RNAseq in a Miseq system (miRNAs).Canonical discriminant analysis (CDA) was used to identify the molecular profile (miRNA and/or proteins) in the exosome cargo from PLWH with NADCs compared to the other study groups.

III. Results

- The baseline clinical and demographic characteristics as well as the plasma exosome concentrations of the HIV⁺NADCs⁻, HIV⁻NADCs⁺, HIV⁺NADCs⁺, and HC groups are shown in **Table 1.**
- A total 599 miRNAs and 317 proteins were detected in the exosomes cargo of study groups. Only those proteins or miRNAs showing differential expression (DE) between the different study groups (130 miRNAS and 133 proteins) were included in the CDA.
- The CDA was able to completely (100%) discriminate between the four study groups **(Figure 2)(Table 2).**

Table 1. Characteristics of the study groups

Characteristic	Study Group				p-value
	HIV⁻NADC⁺	HIV⁻NADC⁺	HIV⁺ NADC⁻	HC	
n	11	10	11	10	NA
Age (years)	59 [48-65]	56 [46-68]	54 [50-60]	58 [52-64]	0.949
Sex (% males)	100	90	73	100	0.077
CD4⁺ counts (cells/µL)	345 [273-572]	NA	609 [496-828]	NA	0.061
Years on ART	19 [9-25]	NA	13 [8-18]	NA	0.549
Plasma exosome concentration (EVs/mL)	1.10 x 10^{11} [6.5 x 10^{10} - 7.5 x 10^{11}]	1.10 x 10^{11} [7.6 x 10^{10} - 2.2 x 10^{11}]	9.75 x 10^{10} [5.5 x 10^{10} - 1.9 x 10^{11}]	1.50 x 10^{11} [7.8 x 10^{10} - 3.4 x 10^{11}]	0.606

Data are given as median [IQR], except sex that is expressed as percentage. Statistical differences between the four study groups were tested by Kruskal-Wallis test for continuous variables and by Chi-square test for sex. Statistical significant was considered for p-values below 0.05; NA: not apply; EVs: Extracellular Vesicles=Exosomes; ART: antiretroviral treatment

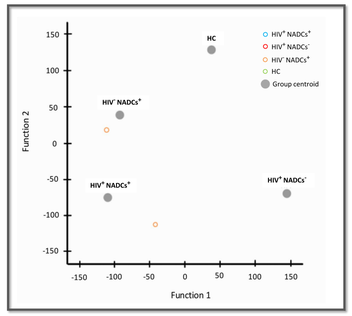


Figure 2. Scatter Plot showing the distribution of the different study groups according to the canonical discriminant functions. The two-dimensional plot shows the centroids of each study group for the discriminant functions 1 and 2. Group centroid: mean value of each discriminant functions for each group.

Table 2. Variables identified by the CDA model discriminating between all the study groups (HIV⁺NADCs⁻, HIV⁻NADCs⁺, HIV⁺NADCs⁺, HC).

		Function	
		1	2
Proteins	IGLV3-10	-5,006	-2,680
	IGKV2D-30	-2,795	-2,338
	SDCBP	-11,655	4,275
	IGLV3-27	1,673	-0,858
	LRG1	-0,689	2,811
	RBP4	-5,667	-1,892
	FTL	11,549	5,159
	IGHV1-8	9,752	5,115
	IGFBP3	6,791	8,923
	S100A7	-11,021	-24,528
	CASP14	0,923	0,466
	HBG1	-12,559	-1,035
	HBA1	1,442	4,839
	HGFAC	2,013	4,431
	FCN2	-26,831	9,023
	KRT78	6,595	11,058
miRNAs	miR-145-3p	-3,907	-4,212
	miR-181c-3p	8,001	-10,615
	miR-3138	25,512	1,579
	miR-337-3p	6,498	-0,104
	miR-4659a-3p	5,868	14,677
	miR-4659a-5p	15,728	-7,603
	miR-520h	0,805	-5,436
	miR-526b-5p	-3,925	3,829
	miR-548ac	-2,519	4,448
	miR-744-3p	9,309	-3,884
	miR-154-p18-3p	-10,422	11,503
	miR-mizuguchi-124	-9,845	10,596

- The CDA for pairs of groups (six different models) was also able to perfectly (100%) discriminate between participants in each group. Moreover, the profile of miRNAs and proteins included in each of the six CDA models was exclusive of each model, suggesting a specific profile of exosomes cargo associated to each study group (**Tables 3-8**).
- Interestingly, the CDA models discriminating between HIV⁺NADCs⁺ and HIV⁻NADCs⁺ or between HIV⁺NADCs⁺ and HIV⁺NADCs⁻ included different miRNAs and proteins involved in the promotion and progression of cancer (i.e. miR-1298-5p, miR-135b-5p, glutathione peroxidase 3 (GPX3), retinol-binding protein 4 (RBP4), collagen alpha-1(VI) chain (COL6A1), dermcidin (DCD)) (**Tables 3 and 4**).

Table 3. Variables identified by the CDA model discriminating between the HIV⁺NADCs⁺ group and the HIV⁻NADC⁺ group

		Function
		1
Proteins	IGKV2D-30	3,969
	IGKV2-24	24,497
	IGKV2-30	14,764
	GPX3	28,711
	HBG1	16,680
	DCD	-9,721
miRNAs	miR-135b-5p	-5,639

Table 4. Variables identified by the CDA model discriminating between the HIV⁺NADCs⁺ group and the HIV⁺NADCs⁻ group

		Function
		1
Proteins	IGKV1-27	2,697
	IGKV2-40	5,584
	RBP4	0,904
	IGLC3	3,294
	COL6A1	25,589
	HBG1	31,102
miRNAs	miR-1298-5p	13,216
	ZC3HAV1-miR	-27,943

Table 5. Variables identified by the CDA model discriminating between the HIV⁺NADCs⁺ group and the HC group

		Function
		1
Proteins	IGKV1-33	28,916
	S100A8	24,367
	GSN	-2,294
	HSPA1A	-28,462
	CETP	16,492
	CDH5	3,197
	UBA52	13,258
	FCN2	-24,220
miRNAs	miR-34c-5p	25,377
	miR-4661-5p	7,776
	miR-744-3p	-1,321
	miR-154-p18-3p	-9,003

Table 6. Variables identified by the CDA model discriminating between the HIV⁻NADCs⁺ group and the HIV⁺NADCs⁻ group

		Function
		1
Proteins	IGKV1-27	22,413
	IGKV1D-13	4,535
	IGKV2-24	4,478
	SDCBP	10,280
	HBG1	30,947
	FCN2	21,237
miRNAs	miR-1304-5p	7,247
	miR-4669	2,358
	miR-5683	13,684
	miR-7848-3p	-2,015

Table 7. Variables identified by the CDA model discriminating between the HIV⁻NADCs⁺ group and the HC group

		Function
		1
miRNAs	IGHV3-30	1,973
	S100A8	16,092
	HSPA8	31,495
	MMRN1	10,858
	SPARCL1	21,144
Proteins	miR-11401	1,167
	miR-122-3p	10,795
	miR-6510-3p	-4,058
	miR-766-5p	-7,170

Table 8. Variables identified by the CDA model discriminating between the HIV⁺NADCs⁻ group and the HC group

		Function
		1
Proteins	IGKV2-24	-3,972
	SERPINA3	-10,349
	RBP4	29,857
	SHBG	16,118
	PRSS3	9,870
miRNAs	miR-1224-5p	-1,339
	miR-429	7,630
	miR-4669	1,996
	miR-548k	3,801
	miR-607	28,047
	miR-6516-5p	3,487
	QXBT11	8,248

IV. Conclusions

Our results show the existence of a specific exosomes cargo associated to concomitant HIV infection and NADCs characterized by increased levels of pro-oncogenic and decreased levels of anti-oncogenic proteins and miRNAs. These results show the existence of a relevant role of HIV infection in the promotion and progression of cancer.

V. Fundings/Acknowledgement

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