Specific exosomal proteomic profile associated to PLWH presenting NADCs

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I. Background

• People living with HIV have an increased risk of developing different types of cancers (NADCs: Non-Aids Defining Cancers). However, it is poorly understood the underlying mechanisms that link up HIV infection with the development and progression of these cancers. Previous *in vitro* studies have suggested that exosomes derived from HIV infected cells can promote and enhance cancer.

• Herein, we have analyzed the proteomic profile of exosomes from HIV patients with active NADC to identify biomarkers associated to this condition.

II. Methods

• Forty-five HIV patients were included: 15 on cART (HIV$^+$NADC$^-$),15 on cART with NADC (HIV$^+$NADC$^+$) and 15 HIV-uninfected with NADCs (HIV$^-$NADC$^+$). Fifteen healthy volunteers (HC) were included as reference.

• For each subject, exosomes were isolated from plasma by Size Exclusion Chromatography, and were quantified and visualized by Nano Tracking Analysis (Nanosight) and Transmission Electronic Microscopy (TEM).

• Proteins were extracted from exosomes, digested with trypsin and the resulting peptides were analyzed by liquid chromatography coupled with mass spectrometry. Proteins identification was carried out using the Mascot search engine through the Protein Discoverer software.

• Differential abundance of proteins between study groups was considered when the ratio of abundance was ≥2 or ≤0.5

Table 1. Characteristics of subjects included in the study.

Characteristic		Study Group				p-value[1]	p-value[2]	p-value[3]	p-value[4]
		HIV$^+$NADC$^-$	HIV$^+$NADC$^+$	HIV$^-$NADC$^+$	HC				
n		15	15	15	15	NA	NA	NA	NA
Age (years)		52 [49-59]	57[49-66]	52[46-65]	56[50-63]	0.774	0.692	0.406	0.561
% Male		73	100	87	87	0.202	0.099	0.032	0.143
CD4 counts (cells/µL)		609 [416-840]	450 [322-764]	NA	NA	NA	NA	0.138	NA
Time since HIV-diagnosis (years)		16 [12-18]	20 [14-29]	NA	NA	NA	NA	0.174	NA
Lenght of HIV-treatment (years)		15 [12-18]	17 [10-20]	NA	NA	NA	NA	0.782	NA
Type of cancer (%)	Lung	NA	26.7	26.7	NA	NA	NA	NA	1.000
	Anal	NA	6.7	6.7	NA	NA	NA	NA	
	Liver	NA	13.3	13.3	NA	NA	NA	NA	
	Kidney	NA	6.7	6.7	NA	NA	NA	NA	
	Pancreas	NA	6.7	6.7	NA	NA	NA	NA	
	Gastric	NA	6.7	6.7	NA	NA	NA	NA	
	Prostate	NA	20	20	NA	NA	NA	NA	
	Head-neck	NA	6.7	6.7	NA	NA	NA	NA	
	Brain	NA	6.7	6.7	NA	NA	NA	NA	
Metastasis (%)		NA	53	60	NA	NA	NA	NA	0.713
Cancer treatment (%)		NA	87	87	NA	NA	NA	NA	1.000

Data are given as median [IQR], except sex, type of cancer, metastasis and proportion of patients on cancer treatment, expressed as %; p-value1: comparison between four study groups (HIV$^+$NADC$^-$, HIV$^+$NADC$^+$, HIV$^-$NADC$^+$, HC; Kruskal Wallis test); p-value2: comparison between HIV$^+$NADC$^-$, HIV$^+$NADC$^+$ and HIV$^-$NADC$^+$ groups (Kruskal Wallis test); p-value3: comparison between HIV$^+$NADC$^-$ and HIV$^+$NADC$^+$ groups (Mann Whitney test); p-value4: comparison between HIV$^+$NADC$^-$ and HIV$^-$NADC$^+$ groups (Mann Whitney test). Sex, type of cancer, metastasis and proportion of patients on cancer treatment comparisons were evaluated with $\chi 2$ test. NA: not apply.

III. Results

- PCAs analysis show clear differences in proteomic pattern between patients and healthy subjects regardless of patient´s group: HIV$^-$NADC$^+$ (**Figure 1A**), HIV$^+$NADC$^-$ (**Figure 1B**) and HIV$^+$NADC$^+$ (**Figure 1C**). More interesting, PCA analysis highlights important differences in proteomic profile between HIV$^+$NADC$^+$ and HIV$^+$NADC$^-$ groups (**Figure 2**). The PC1 component was dominant in all the cases to separate between groups.
- Most identified proteins in all patient´s groups (HIV$^-$NADC$^+$, HIV$^+$NADC$^-$ and HIV$^+$NADC$^+$) were differentially expressed compared to HC group. Interestingly, remarkable proteins related to cancer development and progression (i.e., hQSOX, proprotein convertase 9, complement component C9, and c1r-LP among others) were up-regulated in the HIV$^+$NADC$^+$ group (**Table 2**). In contrast, one of the proteins down-regulated in HIV$^+$NADC$^+$ patients is considered as anti-oncogenic factor (protein GPx-3) (**Table 2**), and curiously, its mRNA expression is downregulated by HIV-Tat. Of note, several proteins were exclusive of HIV$^+$NADC$^+$ vs HC comparison, of which some were up-regulated in HIV$^+$NADC$^+$ group.
- Almost half of the total identified proteins in the HIV$^+$NADC$^+$ and HIV$^+$NADC$^-$ comparison were differentially expressed between these groups of patients. Notably, more than 40% of them were up-regulated in HIV$^+$NADC$^+$ group. In addition, there were some proteins exclusive of the comparison between HIV$^+$NADC$^+$ and HIV$^+$NADC$^-$, including two proteins (SAA1 and Thrombospondin 1 proteins) associated with the development of cancer and metastasis that were upregulated in NADC$^+$HIV$^+$ (**Table 3**). Interestingly, SAA1 mRNA expression is upregulated by HIV-gp120, showing the potential role of HIV as trigger of cancer development and progression.

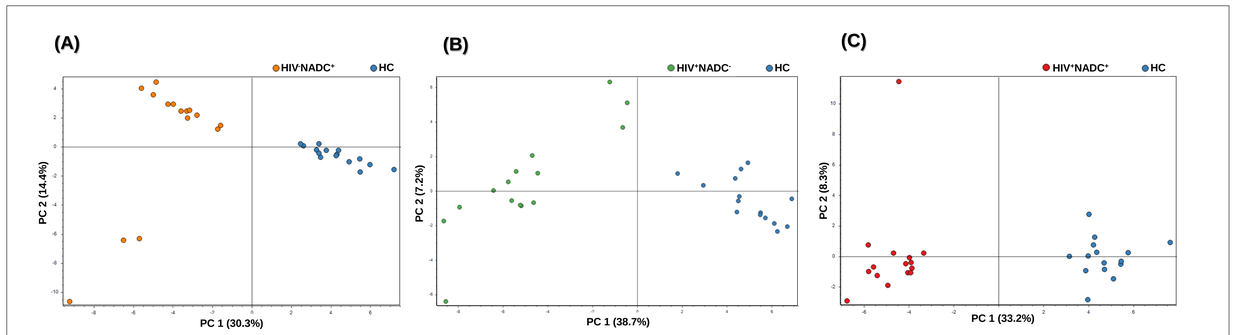


Figure 1. Principal component analysis (PCA) comparing the proteomic profile of exosomes between patients from different study groups and healthy controls (**blue** dots): (**A**) HIV$^-$NADC$^+$ patients (**orange** dots); (**B**) HIV$^+$NADC$^-$ patients (**green** dots); and (**C**) HIV$^+$NADC$^+$ (**red** dots).

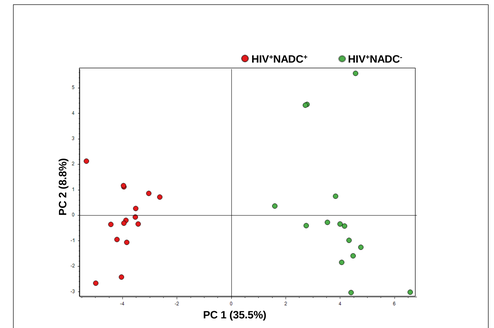


Figure 2. Principal component analysis (PCA) comparing the proteomic profile of exosomes between HIV$^+$NADC$^+$ (**red** dots) and HIV$^-$NADC$^+$ (**green** dots) study groups of patients.

Table 2. Remarkable cancer-related proteins differentially expressed between HIV$^+$NADC$^+$ and HC groups

	Protein	Description
Up-regulated in HIV$^+$ NADC$^+$ group	O00391	Sulfhydryl oxidase 1 (hQSOX), involved in regulation of transport and uptake of the insulin-like growth factor-I, associated to cancer promotion
	Q8NBP7	Proprotein convertase subtilisin/kexin type 9 (PCSK9), associated with proliferation, migration and invasion of breast cancer and its overexpression has been associated with oncogenesis
	P02748	Complement component C9, increased in squamous cell lung cancer
	Q9NZP8	Complement C1r subcomponent-like protein (C1r-LP), proposed as markers of several cancers (hepatocellular carcinoma, glioblastoma and kidney cancer
Down-regulated in HIV$^+$NADC$^+$ group	P22352	Glutathione peroxidase 3 (GPx-3), a plasma antioxidant, anti-oncogenic factor whose downregulation has been associated with several cancers (thyroid cancer, hepatocellular carcinoma and myeloid leukemia)
	P02652	Apolipoprotein A-II, involved in chylomicron assembly and remodeling, HDL remodeling and retinoid metabolism and transport. Also, it has been proposed as early marker of pancreatic cancer

Table 3. Remarkable cancer-related proteins exclusively found in the comparison between HIV+NADC+ and HIV+NADC- groups

	Protein	Description
Up-regulated in HIV$^+$NADC$^+$ group	P0DJI8	Serum amyloid A-1 protein (SAA1), contributes to cancer development and accelerates tumor progression and distant metastasis
	P07996	Thrombospondin-1 (TSP1), proposed as a biomarker of the pathological malignancy of glioma, correlated with tumor immune microenvironment in glioblastoma
	P18428	Lipopolysaccharide-binding protein (LBP), involved in Toll Like Receptor 4 cascade, in the transference of LPS, and in interleukin-4 and interleukin-13 signaling

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V. Conclusions

- Our results show the existence of a specific exosomal proteomic profile associated with concomitant HIV infection and NADCs, characterized by increased levels of proteins related to cancer promotion and reduced levels of anti-oncogenic factors.
- Of note, some of these proteins are considered HIV-regulated targets, supporting the potential role of HIV in triggering cancer development and progression.