# VIRAL AND HOST PROTEINS CARRIED BY EXOSOMES FROM PLWH COULD PLAY A ROLE AS TRIGGERS OF NADCS

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## I. Background

- A higher incidence and more rapid progression of several types of non-AIDS-defining cancer (NADC) have been reported in people living with HIV (PLWH). However, the exact mechanism linking HIV-1 infection to the development of NADCs is not yet fully understood.
- *In vitro* studies have suggested that viral molecules carried by exosomes derived from HIV-1-infected cells can promote and exacerbate cancer.
- Herein, we analysed the proteomic (viral and host) profile of exosomes from patients with active NADC, stratified according to the presence or absence of HIV infection.

## II. Methods

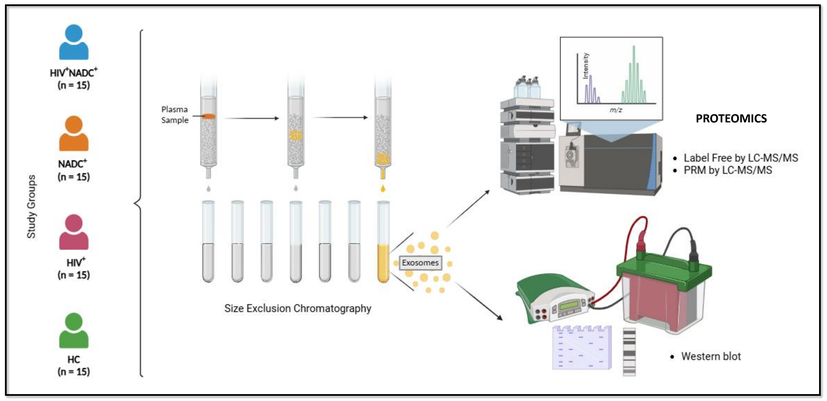



**Figure 1. General outline of the methodology.** 4 study groups were included: 15 PLWH on ART with NADC (HIV⁺NADC⁺ group), 15 people with NADC without HIV-1 (NADC⁺ group), 15 PLWH on cART without NADC (HIV⁺ group) and 15 healthy volunteers (HC group). A non-targeted label-free quantitative proteomic by LC-MS/MS, parallel reaction monitoring (PRM-MS/MS), and western blot analyses were performed in exosomes purified by Size Exclusion Chromatography (SEC) from plasma of all study subjects.

## III. Results

**Table 1. Characteristics of the study groups**

| Characteristic | HIV⁺NADC⁺ (n=15) | NADC⁺ (n=15) | HIV⁺ (n=15) | HC (n=15) | p-value |
|---|---|---|---|---|---|
| **Age (years)** | 57 [48-65] | 52 [46-65] | 52 [49-59] | 56 [50-63] | 0.774 |
| **Sex (% males)** | 100 | 87 | 73 | 87 | 0.501 |
| **CD4⁺ counts (cells/μL)** | 485 [309-771] | NA | 630 [496-828] | NA | 0.101 |
| **Time since HIV diagnosis (years)** | 25 [15-28] | NA | 16 [12-18] | NA | 0.065 |
| **Years on ART** | 15 [9-21] | NA | 14 [9-18] | NA | 0.948 |
| **Plasma exosome concentration (EVs/mL)** | $9.50 \times 10^{10}$ [$6.0 \times 10^{10}$ - $3.8 \times 10^{11}$] | $9.00 \times 10^{10}$ [$1.4 \times 10^{10}$ - $1.5 \times 10^{11}$] | $9.75 \times 10^{10}$ [$5.5 \times 10^{10}$ - $2.3 \times 10^{11}$] | $1.12 \times 10^{11}$ [$5.9 \times 10^{10}$ - $2.9 \times 10^{11}$] | 0.595 |

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

## III. Results

- Forty human proteins were found differentially expressed (DE) in the exosomes of the HIV⁺NADC⁺ group compared to the NADC⁺ group. A distinctive proteomic profile of the exosomes in these groups is observed in **Figure 2.**
- Functional enrichment analysis in this comparison showed that innate immune response pathway was enriched in upregulated proteins, and adaptive immune response ontology term was enriched in downregulated proteins (adjusted p-value<0.05) **(Figure 3).**

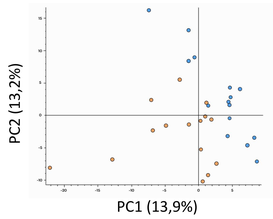



**Figure 2. Principal component analysis (PCA) plot** showing the distribution of the HIV⁺NADC⁺ (blue) and NADC⁺ (orange) study groups based on the proteomic profile of their exosomes.

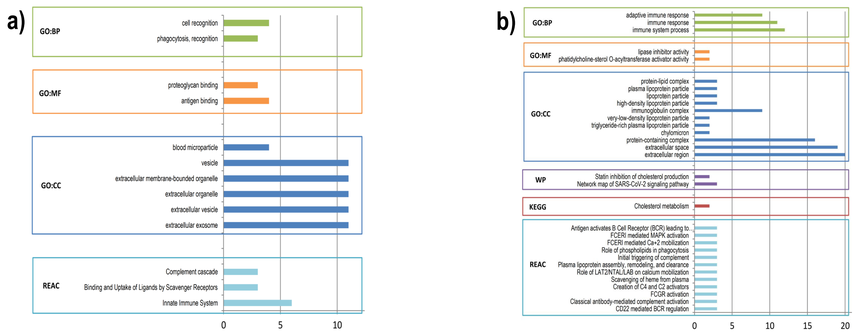



**Figure 3. Functional enrichment analysis of the a) upregulated and b) downregulated proteins from exosomes between HIV⁺NADC⁺ group and NADC⁺ group**. Functional terms from different databases are represented: Gene Ontology (Biological Function/GO:BF, Molecular Function/GO:MF, and Cellular Complex/GO:CC); Reactome (REAC); Kyoto Encyclopedia of Genes and Genomes (KEGG); and WikiPathways (WP).

- On the other hand, gp120, IN and RT HIV-1 viral proteins were detected in the exosomes of the HIV⁺NADC⁺ group and the HIV⁺ group **(Figure 4).**
- Interestingly, the gp120 protein was increased in HIV⁺NADC⁺ group compared to HIV⁺ group ($p<0.05$), the IN protein was decreased in HIV⁺NADC⁺ group compared to HIV⁺ group ($p<0.05$) while the RT protein did not show differences between both groups **(Figure 4).**
- Of note, both gp120 and RT proteins, have previously been associated with cancer events.

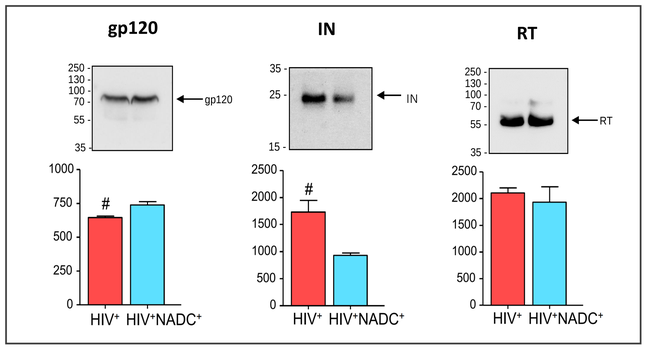



**Figure 4. Western blot gel images and bar graphs** showing the detection and quantification of HIV-1 viral proteins from plasma exosomes of subjects in the HIV⁺NADC⁺ and HIV⁺ groups. gp120: Envelope glycoprotein 120; IN: Integrase protein; RT: Retrotranscriptase protein; #: t-test $p<0.05$

- Remarkably, among the DE proteins found in the comparison between the HIV⁺NADC⁺ group and the NADC⁺ group, five pro-oncogenic (QSOX1, COMP, FTL, OIT3, KRT6B) proteins were upregulated and three anti-oncogenic (HGFAC, GPX3, and SPARCL1) were downregulated in the HIV⁺NADC⁺ group **(Table 2).**
- Moreover, six of these eight proteins were also DE in HIV⁺ group compared to the NADC⁺ group in the same direction as the HIV⁺NADC⁺ versus NADC⁺ groups.
- Thus, these findings may indicate that HIV infection is capable of modulating the expression of oncogenic proteins, thereby contributing to the development of NADCs.

**Table 2. Cancer-related exosomal proteins differentially expressed in the comparison HIV⁺NADC⁺ vs. NADC⁺ groups**

| | Protein ID | Description |
|---|---|---|
| **Upregulated in HIV⁺NADC⁺ group** | O00391 | Sulfhydryl oxidase 1 (**QSOX1**), involved in regulation of transport and uptake of the insulin-like growth factor-1, associated to cancer promotion. It is also involved in development, metastasis and survival in several cancers such us lung cancer, breast cancer, prostate cancer, pancreatic cancer, neuroblastoma, and glioblastoma. |
| | P49747 | Cartilage oligomeric matrix protein (**COMP**), a tumour microenvironment regulator, involved in differentiation and stage of various cancers. In particular, it has been associated to colorectal cancer and gastric and esopharingeal adenocarcinoma. |
| | P02792 | Ferritin light chain (**FTL**), the L-subunit of ferritin protein, is responsible for intercellular iron storage. It has been found up regulated in patients with mesothelioma, colorectal cancer, gastric cancer, breast cancer, prostate cancer and glioma among others. |
| | P04259 | Keratin type II cytoskeletal 6B (**KRT6B**), a protein from the keratin family, associated with metastasis in bladder cancer. Also found in exosomes of bladder cancer patients related to immune response. |
| | Q8WWZ8 | Oncoprotein-induced transcript 3 (**OIT3**), involved in hepatocellular function and development, mediates macrophage polarization and facilitates hepatocellular carcinoma progression. |
| **Downregulated in HIV⁺NADC⁺ group** | Q04756 | Hepatocyte growth factor activator (**HGFAC**), proposed as a useful and predictive biomarker for hepatic cancer. |
| | P22352 | Glutathione peroxidase 3 (**GPX3**), a plasma antioxidant, and an anti-oncogenic factor whose down regulation has been associated with several cancers (thyroid, hepatocellular, breast and myeloid leukemia). |
| | Q14515 | SPARC-like protein 1 (**SPARCL1**), a member of the SPARC family of extracellular matrix-associated proteins, is a widely known |

## IV. Conclusions

The presence of oncogenic HIV-1 proteins and several other cancer-related proteins from the host carried by exosomes in people living with both HIV and NADCs implies that HIV infection might be a significant factor in the dysregulation of “onco-proteins” influencing a poorer prognosis in those patients with NADCs.

## V. Fundings/Acknowledgement

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