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PLASMA EXOSOMES FROM INDIVIDUALS WITH HIV AND CANCER ENHANCE TUMOR CELL PROLIFERATION AND MIGRATION

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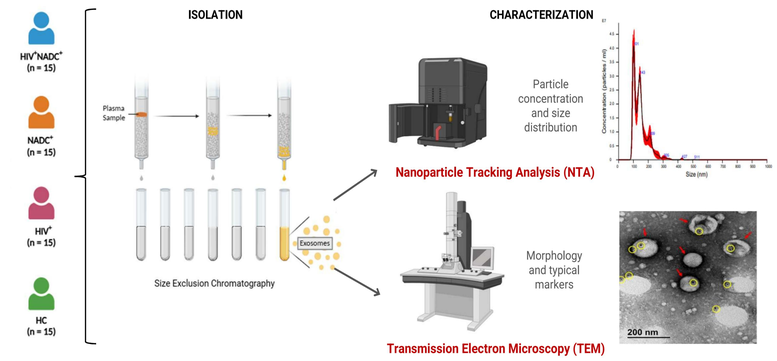
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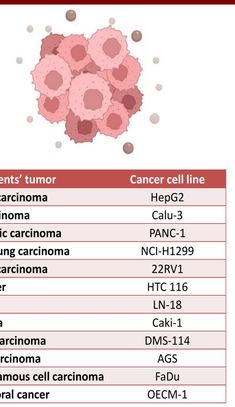
Becas Gilead a la Investigación Biomédica

I. Background

- People living with HIV (PLWH) achieve near-normal life expectancy with combination antiretroviral therapy (cART); nonetheless, they exhibit a higher incidence of non-AIDS-defining cancers (NADCs) than the non-HIV-infected population.
- The mechanisms linking HIV infection to NADC development remain poorly understood. Exosomes have emerged as potential mediators of this HIV-cancer axis as both HIV-infected and tumor cells release vesicles could carry pro-oncogenic cargo.
- We investigated the functional role of plasma exosomes from PLWH with cancer in promoting tumor proliferation and migration.

II. Methods





Patients' tumor	Cancer cell line
Hepatocellular carcinoma	HepG2
Lung adenocarcinoma	Calu-3
Ductal pancreatic carcinoma	PANC-1
Non-small cell lung carcinoma	NCI-H1299
Prostate adenocarcinoma	22RV1
Colorectal cancer	HTC 116
Glioblastoma	LN-18
Renal carcinoma	Caki-1
Small cell lung carcinoma	DMS-114
Gastric adenocarcinoma	AGS
Pharyngeal squamous cell carcinoma	FaDu
Squamous cell oral cancer	OECM-1

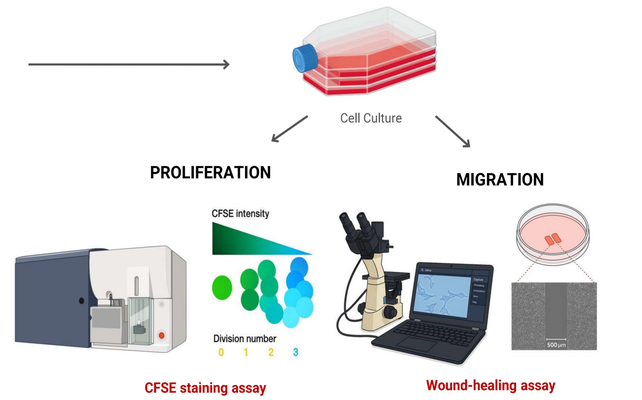


Figure 1. General outline of the methodology. Sixty individuals were enrolled and divided into four groups: 15 PLWH on cART with NADC (HIV⁺NADC⁺), 15 HIV-uninfected with NADC (NADC⁺), 15 PLWH on cART without NADC (HIV⁺), and 15 healthy controls (HC). HIV⁺NADC⁺ and NADC⁺ groups were matched for cancer type to minimize tumor-specific bias. Plasma exosomes were purified by size-exclusion chromatography and characterized using transmission electron microscopy (negative staining and immunogold labeling) and nanoparticle tracking analysis to confirm size distribution and structural integrity. Functional assays employed cancer cell lines representative of each patient's tumor type. Cell proliferation was assessed by CFSE staining and migration by wound-healing assays at multiple time points to capture dynamic changes.

III. Results (a)

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)	9.50×10^{10} [6.0×10^{10} - 3.8×10^{11}]	9.00×10^{10} [1.4×10^{10} - 1.5×10^{11}]	9.75×10^{10} [5.5×10^{10} - 2.3×10^{11}]	1.12×10^{11} [5.9×10^{10} - 2.9×10^{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. Statistically significant was considered for p-values below 0.05; NA: not apply; EVs: Extracellular Vesicles=Exosomes; ART: antiretroviral treatment.

III. Results (b)

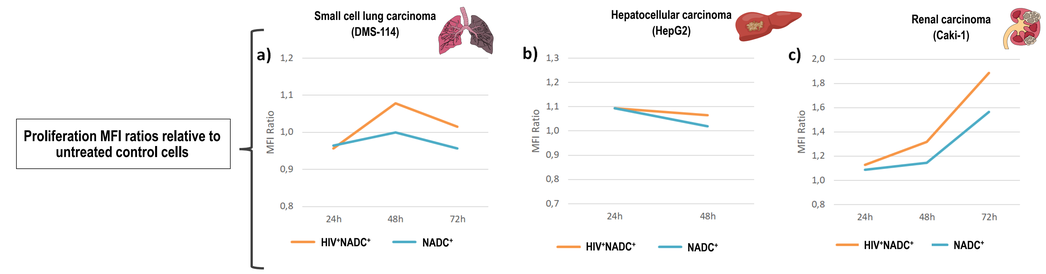


Figure 2. Proliferation MFI ratios relative to untreated control cells of: (a) DMS-114, (b) HepG2, (c) Caki-1 cancer cell lines after exposure to plasma exosomes from the HIV⁺NADC⁺ group and from the NADC⁺ group. Each colored line represents the proliferation observed for each individual subject. The x-axis indicates the time intervals of exosomes treatment applied to the corresponding cancer cell lines. MFI: Mean Fluorescence Intensity.

Plasma exosomes from people with HIV and NADC induced stronger tumor cell proliferation than exosomes from people with only NADC: In lung cancer (DMS-114 cell line) proliferation increased by 13%, in hepatocellular carcinoma (Hep G2 cell line) by 16%, and in renal cancer (Caki-1 cell line) by up to 20% (**Figure 2**).

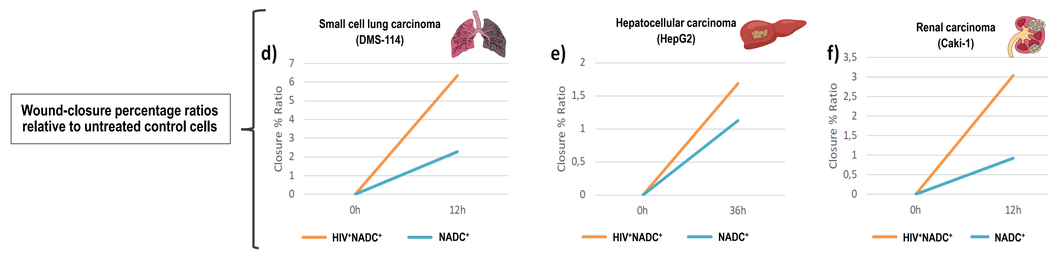


Figure 3. Wound-closure percentage ratios relative to untreated control cells of: (a) DMS-114, (b) HepG2, (c) Caki-1 cancer cell lines after exposure to plasma exosomes from the HIV⁺NADC⁺ group and from the NADC⁺ group. Each colored line represents the migration observed for an individual subject. The x-axis indicates the time intervals of exosomes treatment applied to the corresponding cancer cell lines.

Plasma exosomes from people with HIV and NADC induced stronger tumor cell migration than exosomes from people with only NADC. In lung cancer (DMS-114 cell line) migration increased up to 3.5-fold, in hepatocellular carcinoma (Hep G2 cell line) by nearly 1.5-fold, and in renal cancer (Caki-1 cell line) by 3.3-fold (**Figure 3**). (See also **Figure 4** for representative images of the migration assay).

III. Results (c)

In parallel of this study, a complementary study was performed to analyze the proteomic and transcriptomic exosomal cargo from the same study groups. It was found that exosomal cargo from people with HIV and NADC have: upregulation of pro-oncogenic proteins and ncRNAs (QSOX1, KRT6B, COMP, SDCBP, and SNORD3A), and downregulation of anti-oncogenic proteins and ncRNAs (HGFAC and VTRNA1.1) (**Figure 5.**)

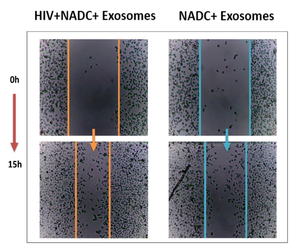


Figure 4. Images of the wound-healing assay showing the closure of the migration gap in AGS cancer cell line at 0 h and 15 h after treatment with plasma exosomes from an HIV⁺NADC⁺ patient (orange) and a NADC⁺ patient (blue).

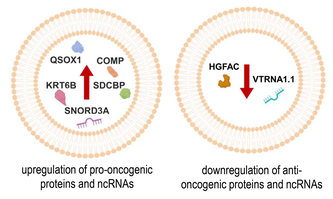


Figure 5. Schematic summary of exosomal cargo alterations in people with both HIV and NADC.

IV. Conclusions

- Plasma exosomes from PLWH with cancer enhance tumor cell proliferation and migration across multiple tumor types.
- These findings support a potential role for exosomes as mediators of tumor progression and underscore their cargo as a promising target for future diagnostic and therapeutic strategies.

V. Fundings/Acknowledgement

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VI. Plain Language Summary

"Messenger particles" (exosomes) from the blood of people living with HIV and cancer made tumor cells grow and move more than those particles from people with cancer who do not have HIV.