Surface Engineering Of Extracellular Vesicles To Target HIV Persistence

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ABSTRACT

Antiretroviral therapy (ART) suppresses HIV replication but fails to eliminate the latent reservoir and achieve complete viral eradication. Hence, there is a profound interest in alternative therapeutic strategies. Extracellular vesicles (EVs) are 30 to 2000 nm sized membrane vesicles and key players of intercellular signaling. They have shown promise as engineerable therapeutic agents for a broad range of diseases, as therapeutic drug carriers and targeted cell delivery systems. Using surface display technology, we surface-decorated EVs with HIV envelope-targeting fusion proteins and potently inhibited HIV infection *ex vivo*. Our work suggests that the designer exosomes may be utilized as delivery nanoshuttles to specifically bind and mediate clearance of HIV-infected cells.

INTRODUCTION

1. Extracellular vesicles (EVs) are secreted by a variety of cells and have immunological and intercellular signaling functions.

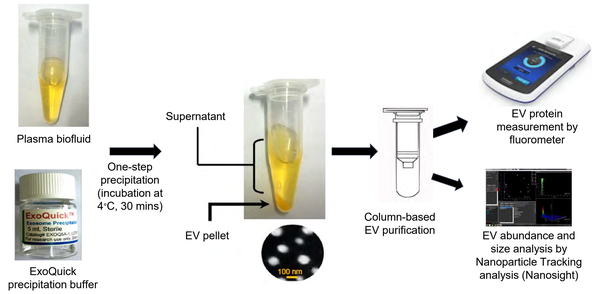
2. EVs can be surface-decorated with chimeric antibody fusion proteins for cell-specific targeting using an exosomal surface display system (XStamp technology, SBI).

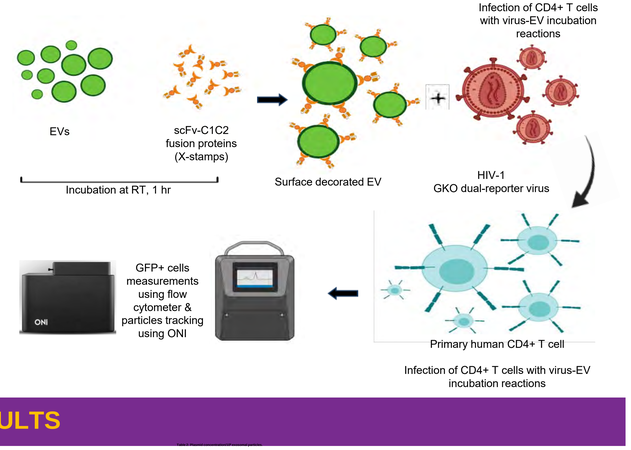
3. The technology is based on C-terminal fusion of desired cell-specific ligands to the C1C2 domain (C-terminal domain of the MFG-E8 protein which exclusively localizes to exosomal membranes).

METHODOLOGY

EVs were isolated from 1 ml of healthy donor plasma using polymer-based precipitation and column purification. EVs were decorated with scFv-C1C2 fusion proteins targeting the HIV envelope protein. The HIV-1 GKO dual-reporter virus (1 µg) was incubated with decorated exosomes for two hours at 1:1, 1:2, and 1:4 ratios at 37°C. Also, virus was incubated with no exosomes, undecorated exosomes, or anti-PD-1 scFv-decorated exosomes (controls). 2*10^6 Jurkat E6.1 cells or primary human anti-cd3/cd28-stimulated CD4+ T cells were infected with virus-exosome preparations via spinoculation, and GFP fluorescence was measured by flow cytometry after 72 hours.

EXPERIMENTAL WORKFLOW





RESULTS

Nanoimaging analysis of labeled, engineered EVs with HIV-1 reporter virus

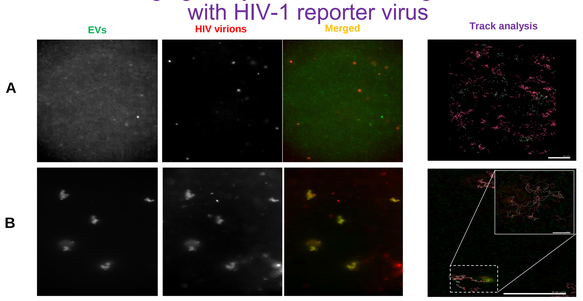


Figure 1. Nanoimaging and single-particle track analysis of labeled EVs (A) non-engineered and (B) anti-HIV envelope surface-engineered incubated with labeled HIV-1 reporter virus using ONI Nanoimager. Surface-engineered EVs and HIV particles were fluorescently labeled using ExoGlow-Protein EV labeling dye and CellBrite cytoplasmic membrane dye. Incubation reactions were visualized in 2-color simultaneously and analyzed by single-particle tracking analysis using a Nanoimager S (ONI) equipped with NimOS software. The HIV-targeting EVs clustered and moved in tandem with virions, in contrast to non-targeting EV controls which tracked independently of virions.

HIV-1 reporter virus infectivity levels in the presence of surface-engineered EVs

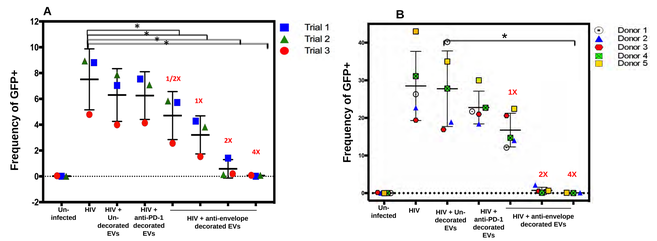
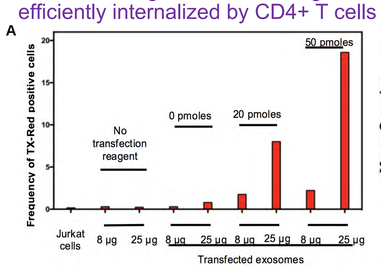
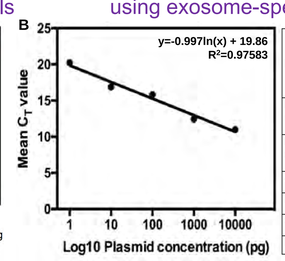


Figure 2. HIV-1 GKO reporter virus infectivity levels in the presence of surface-engineered EVs in (A) Jurkat E6.1 and (B) primary human CD4+ T cells. Frequency of GFP+ (productive infection) are reported after infection of cells with incubation preparations of no virus (uninfected negative control), virus with no EVs (HIV positive control), virus with undecorated (non-targeting EVs), anti-PD-1 decorated (non-specific targeting EVs), or anti-HIV envelope decorated EVs. The anti-HIV envelope decorated EVs significantly inhibited (n=3; *p<0.05, paired t-test) HIV infection in a dose-dependent manner in Jurkat cells with respect to negative controls and inhibited up to 98% infection in the absence of toxicity in primary human CD4+ T cells (n=5 healthy blood donors). *p<0.05, paired t-test.

EVs harboring nucleic acid cargo are efficiently internalized by CD4+ T cells



Pro-apoptotic cargo is efficiently loaded into EVs using exosome-specific lipid transfection



EVs	Plasmid concentration per 10^6 EVs
EVs +2 µg plasmid, no transfection reagent	Out of range
Plasmid control (0 µg transfected)	Out of range
A (1 µg plasmid transfected)	4.69 pg
B (2 µg plasmid transfected)	4.35 pg
C (5 µg plasmid transfected)	7 ng
No template PCR control	Out of range

Figure 3: EVs can efficiently package labeled nucleic acid and deliver pro-apoptotic cargo cargo to cells. A. EVs were transfected with 0, 20, and 50 pmoles of TX-Red fluorescently labeled siRNA. 8 and 25 µg of transfected EVs were incubated with Jurkat cells (10^5 cells/ml) and number of cells positive for TX-Red fluorescence were measured using flow cytometry after 48 hr. The frequency of TX-Red fluorescent cells (y-axis) was higher in cells incubated with transfected EVs (20 and 50 pmoles) as compared to non-transfected EVs, no siRNA, and only cells controls. **B.** A standard curve using 4.5 kb caspase-expression plasmid DNA template (1-10,000 pg) in quantitative PCR was generated. Mean CT values (y-axis) were plotted against the plasmid concentration (log10 scale, x-axis). 1 µl of transfected EVs was used for the PCR reaction to measure plasmid amounts transfected. Utilizing this standard curve, we quantified the plasmid concentrations transfected per 10^6 EV particles Presence of the plasmid DNA was detected in EVs transfected with 1, 2, and 5 µg plasmid. No plasmid DNA was detected in all other controls.

RESULTS

Engineered exosomes specifically target HIV-infected cells

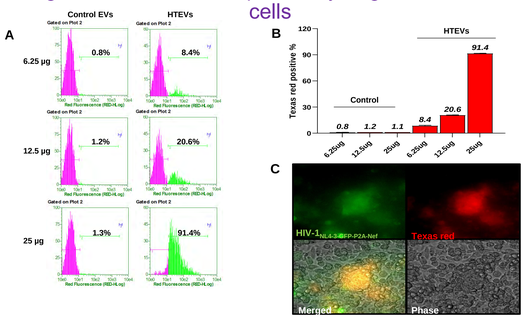


Figure 4. HTEVs are specifically internalized by HIV-infected cells. A and B. Jurkat 2D10 cells were induced with PMA/TSA to reactivate latent $HIV_{NL4\text{-}3\text{-}Dgag/pol\text{-}GFP}$ reporter virus and then were treated with Texas red-labeled control or anti-HIV-C1C2 fusion proteins decorated EVs. After 24h, cells were analyzed by flow cytometry. **C.** TZM-bl cells were *in vitro* infected with $HIV_{NL4\text{-}3P2A\text{-}Nef}$ and two days later were treated with labeled EVs, decorated with C1C2 (control) or anti-HIV-C1C2 proteins. Internalization of decorated EVs was assessed after 18h incubation using fluorescence microscopy. HTEVs efficiently directed EVs into latently-infected, reactivated T lymphoid cells and *in vitro* HIV-1-infected TZM-bl cells.

CONCLUSIONS AND FUTURE WORK

1. Engineered anti-HIV scFv-decorated EVs significantly inhibited HIV infection *ex vivo*.

2. Designer EVs selectively bind to the HIV envelope as observed in dual-color imaging and single-particle tracking using a Nanoimager S (ONI).

3. Future work will test the engineered anti-HIV envelope surface decorated exosomes to accelerate the clearance of the HIV reservoir by:

   - Delivering cytotoxic cargo specifically to infected cells expressing envelope at the cell surface following administration of latency reversal agents.

   - *In vivo* infusion of engineered cells which constitutively secrete cargo-loaded, HIV envelope-targeting EVs.

CONTACT INFORMATION

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