Editorial Commentary

Commentary on "*Exosome-mediated stable epigenetic repression of HIV-1*"

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Extracellular vesicles (EVs) were first described in the 1980s. An area of interest in immunology is the influence of a subset of EVs (exosomes) on the course of human disease and their centrality in the anti-human immunodeficiency virus (HIV) mitigation process. In this commentary, we dissect work published by Shrivastava *et al.*, on exosome-mediated stable epigenetic repression of HIV-1.

While the potency of some antiretroviral agents has captured the interest of researchers and clinicians (1-3), the dangers that lurks clearly in most of these drugs are related to long-term effects and cytotoxicity. For instance, hyperlipidemia, hyperglycemia, and lipodystrophy have been shown to be induced by the well-known highly active antiretroviral therapy (HAART) (3). To navigate this pathological issue, Shrivastava *et al.* postulated that the exosomes derived from the body which contains cellular epigenetic arsenal like DNA methyl transferase 3 alpha (DNMT3A) could on one hand eliminate the problem of cytotoxicity and on the other hand down-regulate HIV-1 transcription (4). Undoubtedly, the progression and pathogenesis of HIV is highly favored by weakening immunity of the infected individuals, hence there is the urgent need to avoid compounding HIV patients with agents and therapies that affect immunity.

Much progress has been made since the discovery and implementation of combination antiretroviral therapy (cART) against HIV infection and control, however, given the complexity of HIV infection and disease progression, new and innovative tools must be employed to navigate HIV disease and latency. In this regard, new innovative strategies have employed the use exosomes to reduce HIV infection. Khatua and group (5) demonstrated that exosomes could release proteins and thus serve as an HIV therapy (5). HIV therapies continue to progress, including promising approaches that attempt to stop a tremendous hurdle, HIV latency and re-emergency. Along these lines, Mousseau *et al.* employed didehydro-cortistatin A (dCA) as a potent Tat inhibitor, reducing HIV-1 transcription and preventing viral rebound. dCA activity demonstrates the potential of the "block-and-lock" functional cure approaches. However, this approach led to dCA resistant virus (6).

Hypermethylation is a well-known genetic method used against most proviruses, for instance, CpG methylation of the human T-cell leukemia virus type 1 (HTLV-1) long terminal repeat (LTR) causes proviral latency (7), the viral 5'LTR cause latency in HIV-1 infection (8). For this reason, targeted DNA methylation of the 5'LTR is a proven method, nonetheless, this method must be rigorously studied against the background of the human body to ensure its safety. Shrivastava *et al.* (4) intelligently established this crucial framework in this study by utilizing a humanize mouse model to recapitulate the human body mechanisms.

The ingenious fusion of zinc finger protein (ZFP362b),

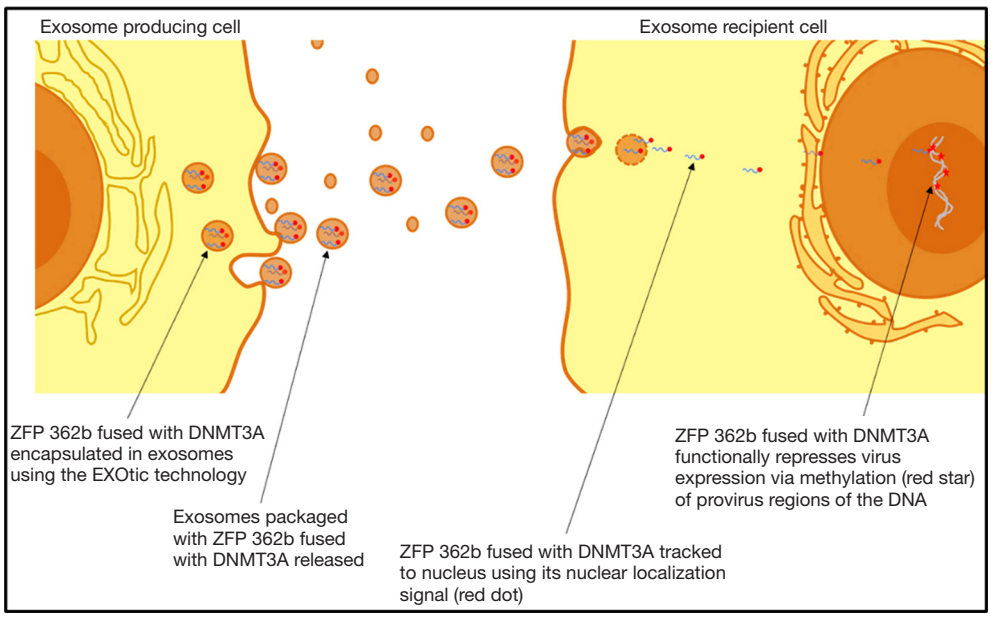

Figure 1 Mechanism of ZFP362b-DNMT3A exosomes payload delivery system using the EXOtic technology. ZFP, zinc finger protein; DNMT3A, DNA methyl transferase 3 alpha; EXOtic, EXOsomal transfer into cells.

which targets and binds to HIV-1 LTR, to the DNA methyl transferase ensures targeted delivery and eradicates the possibility of random methylations processes that can potentiate cascades of deleterious events in the body. Such creativity has immeasurable utility in bioengineering, cancer research and more importantly and most timely, in HIV research (*Figure 1*).

The findings that ZFP362b fused to DNMT3A potently and selectively repressed HIV (4) is huge to HIV vaccinologists and researchers alike (*Figure 1*). To the researcher what catches the eye most was their technique. Exosomes are promiscuous, they pack all kinds of "material" in them. To avoid non-specificity, they adopted the exosome packaging technology devised by Kojima *et al.* (9), coined "EXOtic" (EXOsomal transfer into cells) which targets the exosome markers such as CD63, Alix, and TSG101 which allows for specific recruitment and encapsulation of mRNA into exosomes. Exosomes are released from the producer cell and enter the target cells through membrane fusion (9).

EVs were first described in the 1980s, when they were found in red blood cells (10), reticulocytes (11-13), neoplastic cell lines (14). Exosomes have been considered critical to disease mitigation and progression (15,16). Exosomes are the smallest type of EVs. These endosome-derived small membrane vesicles are approximately 50–150 nm in diameter (9,17-19). Exosome are secreted by cells and released into biological fluids in all living systems (20). An area of interest in immunology is the influence of EVs and exosomes on the course of human disease. Exosomes are central to the development of HIV mitigation and progression (21-23).

The clinical relevance of this interesting work goes beyond HIV repression alone, it represents a myriad of possibilities against several other diseases and cancers. Contrary to most therapeutic systems available which are known to exacerbate the weakening of the patient's immunity, the EXOtic delivery of HIV repressor might significantly reduce viral load in HIV patients and at the same time cause no significant side effect since exosomes are naturally produced and present in the body.

A fundamental challenge in DNA methylation approach is how to direct the methyl transferase into the nucleus initially. The investigators solved this major huddle by embedding a nuclear localization signal into the ZFP362b-DNMT3A construct. They proved via methyl DNA immunoprecipitation that their constructs; ZFP, ZPAMt, and ZKMt were localized in the nucleus and binds to HIV-LTR region. At present, it is unclear which other regulatory factors might be affected by HIV repression using ZFP, and can this technology potentially disrupt any crucial signaling pathways that hamper development and normal tissue homeostasis? What are the long-term effects? More so, we still lack throughput data on the pharmacodynamics and

pharmacokinetics of exosome encapsulated agents and hence this technology suffers similar deficiency. Nonetheless, such an excellent work is highly commendable and paves the way for research into exosomes utility in disease management and more specifically, HIV therapy.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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