Extracellular Vesicles as Enhancers of Virus Vector–Mediated Gene Delivery

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FIG. 1. Electron micrograph of adeno-associated virus serotype 1–producing 293T cells releasing extracellular vesicles. Scale bar: 500 nm.

Abstract

Extracellular vesicles (EVs) being released from two adjacent adeno-associated virus serotype 1 (AAV1)-producing 293T cells are shown by electron microscopy. We have shown that AAV vectors can associate with EVs and enter the media. Furthermore, we have recently reported that EV-associated AAV has robust gene delivery and antibody evasion properties *in vivo*.

EXTRACELLULAR VESICLES (EVs) of either endosomal or plasma membrane origin are small membranous vesicles continuously released by most, if not all, cells. They have emanated as pivotal mediators of intercellular communica-

tion, with capacity to carry specific repertoires of functional proteins and nucleic acids. Naturally capable of traversing biological barriers to transfer genetic information, EVs have more recently emerged as an exciting and novel delivery vehicle for therapeutic prospects.

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Figure 1 shows an electron micrograph of adjacent adeno-associated virus serotype 1 (AAV1)-producing 293T cells releasing EVs into the extracellular environment. One can appreciate the abundance and size heterogeneity of EVs released by the cells. Large cellular processes on the cell on the right appear to be releasing or internalizing EVs. Interestingly, we have shown that AAV endogenously associates with producer cell EVs during standard AAV vector manufacturing, bringing to light that a portion of the viral particles released into the supernatant are naturally enveloped by a lipid bilayer (Maguire *et al.*, 2012; György *et al.*, 2014). We have recently shown that EV-associated AAV (ev-AAV) leads to enhanced transduction *in vivo* and shielding from anti-AAV antibodies (György *et al.*, 2014).

Normally, AAV vectors are recovered by lysing the producer cells, although it has previously been shown that functional viral vector particles can also be found in the culture medium during vector production, and that the extent of viral particles in the media is serotype-dependent (Vandenberghe *et al.*, 2010). While more than one process may certainly be responsible for AAV egress into the media, association with EVs appears to be one of them. With several recent reports demonstrating that other classically defined nonenveloped viruses, including pathogenic hepatitis A virus (Feng *et al.*, 2013), use host-derived cellular membranes to exit cells, it is becoming increasingly clear that EVs play a role in virus shedding in addition to conventional cell lysis. This has implications for how we study viruses and their interactions with cells as well as how they respond to neutralizing antibodies and even vaccines. It also adds a layer of complexity to the historical classification of viruses based on the presence or absence of an envelope, where the virus may also exist with a host lipid bilayer without virally-encoded glycoproteins.

Acknowledgments

All authors would like to acknowledge their mentor, Xandra O. Breakefield, for scientific guidance over the entire course of this research. The authors would also like to thank Maria Ericsson of the Harvard Conventional Electron Microscopy core for the transmission electron microscopy work.

Author Disclosure Statement

C.A.M. and J.S. are inventors on a patent application related to the ev-AAV technology. C.A.M. has a financial interest in Exosome Diagnostics, Inc. C.A.M.'s interests were reviewed and are managed by the Massachusetts General Hospital and Partners HealthCare in accordance with their conflict of interest policies. J.S. is an inventor on several exosome/EV-related patent applications and is now an employee of Exosome Diagnostics, Inc. B.G. and Z.F. have nothing to disclose.

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Received for publication July 11, 2014;
accepted after revision July 24, 2014.

Published online: July 29, 2014.