Extracellular Vesicles Shield Adeno Associated Virus from Neutralization by Pre-Existing Antibody in Human Synovial Fluid While Enhancing Human Osteoarthritic Chondrocyte Transduction

Aysegul Atasoy-Zeybek[1], Ozgun F. Duzenli,[2] Joseph A. Panos[3], Rodolfo E. De la Vega[1,3], George Aslanidi[2], Christopher H. Evans[1]
Department of Physical Medicine and Rehabilitation, Mayo Clinic Rochester, MN, USA[1], The Hormel Institute, University of Minnesota, Austin, MN, USA,[2] Department of Orthopedic Surgery, Mayo Clinic, Rochester, MN, USA[3]
Email of Presenting Author: Atasoy-Zeybek.Aysegul@mayo.edu

Disclosures: A. Atasoy-Zeybek (N), O.F. Duzenli (N), J.A. Panos (N), R.E. De la Vega (N), G. Aslanidi (He holds provisional and issued patents related to AAV vectors technology that have been licensed to various gene therapy companies), C.H. Evans (Genascence Corp.).

INTRODUCTION: Intra-articular injection of therapeutics is an increasingly popular treatment modality for osteoarthritis (OA) but the rapid clearance of therapeutics from the joint after intra-articular injection significantly limits their efficacy. Gene transfer can obviate this problem by engineering articular cells to synthesize anti-arthritic gene products in a sustained fashion. A recent clinical trial confirmed the utility of adeno-associated virus (AAV) in this regard[1]. However, the AAV capsid is antigenic and neutralizing antibodies (Nab) against AAV reduce its transducing ability. The present study evaluated the ability of extracellular vesicles to shield AAV from Nab and thus retain transducing potency. Extracellular vesicles (EVs) are attractive vehicles for this purpose. EVs are well-tolerated by the host immune system and can be engineered to encapsulate therapeutic agents, including viral vectors, to enhance targeting and stability and shield them from Nab. In the present experiments, we have determined whether EV can protect AAV from Nab present in human synovial fluid and whether this has any effect of the transduction of articular chondrocytes, an important target cell for OA gene therapy[2].

METHODS: Naked AAV2.0 and EV-encapsulated AAV2.0 vectors carrying GFP, luciferase or IL-1Ra genes were prepared and characterized. Transduction efficiency was evaluated in primary, human, healthy and osteoarthritic chondrocytes using fluorescence microscopy, luciferase assays and measurement of IL-1Ra levels. To assess Nab resistance, synovial fluid from a patient previously treated with AAV2.5 was used for Nab testing[1]. Naked or EV-AAV vectors were pre-incubated with serial dilutions of the synovial fluid, and the mixtures were then added to HEK293 cells. After overnight incubation, the medium was replaced with growth medium. At 48 hours, transgene expression was quantified by fluorescence intensity and luciferase activity. EV-AAV or naked AAV were injected into rat knee joints, and luciferase expression was monitored using an *in vivo* imaging system (IVIS).

RESULTS: Characterization tests confirmed successful AAV2.0 encapsulation within structurally intact EVs. In the presence of neutralizing human synovial fluid, EV-AAV2.0 maintained robust transgene expression at dilutions greater than 1:10,000 while naked AAV2.0 showed markedly reduced resistance to inhibition (**Figure 1**). EV-AAV2.0 demonstrated significantly higher transduction efficiency compared to naked AAV2.0 in both healthy and osteoarthritic human chondrocytes (**Figure 2**). Notably, osteoarthritic chondrocytes exhibited higher transgene expression levels than healthy chondrocytes when treated with EV-AAV2.0 vectors. IVIS confirmed enhanced vector delivery, with EV-AAV showing significantly higher and more sustained transgene expression in rat knee joints compared to naked AAV controls (**Figure 3**).

DISCUSSION: EV encapsulation preserves AAV2.0 vector integrity while significantly enhancing transduction efficiency and protecting against neutralizing antibodies. The superior performance in osteoarthritic chondrocytes suggests potential therapeutic advantages in diseased cartilage. Notably, EV encapsulation enhances and sustains intra-articular transgene expression in rat knee joints. These findings demonstrate that EV-AAV2.0 complexes represent a promising strategy to overcome limitations imposed by Nab on conventional gene therapy for joint diseases.

SIGNIFICANCE/CLINICAL RELEVANCE: This work establishes EV-encapsulated AAV vectors as an improved gene delivery platform for articular tissues, potentially enabling more effective intra-articular gene therapies for OA while circumventing pre-existing immunity challenges. Future studies will focus on intra-articular injection of EV-AAV-luciferase/IL-1Ra complexes to evaluate real-time *in vivo* cartilage penetration and potential therapeutic efficacy.

REFERENCES: [1]De la Vega, R.E., et al. (2025). A phase 1 clinical trial shows safe, sustained, AAV-mediated expression of IL-1Ra in the human osteoarthritic knee joint. Sci Transl Med 17, PMID: 40465688. [2]Evans et al. (2024) Progress towards a gene therapy of arthritis. JAAOS 32: 1052-1060.

FIGURE 1

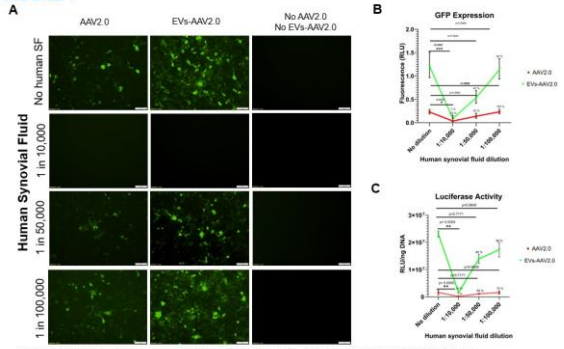

Figure 1. Neutralization of adeno-associated virus (AAV2.0), and extracellular vesicles-encapsulated AAV2.0 (EVs-AAV2.0) by synovial fluid from a patient injected with AAV encoding interleukin-1 receptor antagonist (IL-1Ra) in HEK293 cells. Assessment of neutralization across different dilutions (1–100,000) with **(A)** representative GFP fluorescence images, **(B)** quantification of GFP fluorescence intensity and **(C)** luciferase activity.

FIGURE 3

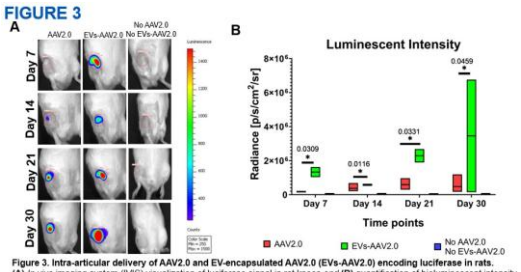

Figure 3. Intra-articular delivery of AAV2.0 and EV-encapsulated AAV2.0 (EVs-AAV2.0) encoding luciferase in rats. **(A)** *In vivo* imaging system (IVIS) visualization of luciferase signal in rat knees *and* **(B)** quantification of bioluminescent intensity.

FIGURE 2

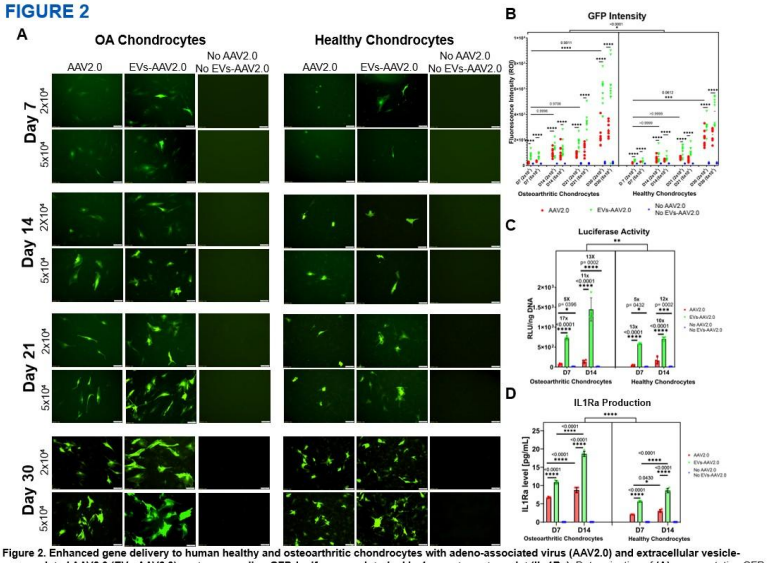

Figure 2. Enhanced gene delivery to human healthy and osteoarthritic chondrocytes with adeno-associated virus (AAV2.0) and extracellular vesicle-encapsulated AAV2.0 (EVs-AAV2.0) vectors encoding GFP, luciferase, or interleukin-1 receptor antagonist (IL-1Ra). Determination of **(A)** representative GFP fluorescence images at days 7, 14, 21, and 30 showing dose-dependent ($2x10^4$; $5x10^4$ vg/cell) expression, **(B)** quantification of GFP fluorescence intensity across vector doses over time, **(C)** luciferase activity measurements at the dose of $2x10^4$ vg/cell, and **(D)** levels of IL-1Ra protein production at the dose of $2x10^4$ vg/cell.