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HISTONE DEACETYLASE INHIBITION ENHANCES ADENOVIRAL VECTOR TRANSDUCTION IN INNER EAR TISSUE

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Abstract—Adenovirus vectors (AdVs) are efficient tools for gene therapy in many tissues. Several studies have demonstrated successful transgene transduction with AdVs in the inner ear of rodents [Kawamoto K, Ishimoto SI, Minoda R, Brough DE, Raphael Y (2003) J Neurosci 23:4395–4400]. However, toxicity of AdVs [Morral N, O'Neal WK, Rice K, Leland MM, Piedra PA, Aguilar-Cordova E, Carey KD, Beaudet AL, Langston C (2002) Hum Gene Ther 13:143–154.] or lack of tropism to important cell types such as hair cells [Shou J, Zheng JL, Gao WQ (2003) Mol Cell Neurosci 23:169–179] appears to limit their experimental and potential clinical utility. Histone deacetylase inhibitors (HDIs) are known to enhance AdV-mediated transgene expression in various organs [Dion LD, Goldsmith KT, Tang DC, Engler JA, Yoshida M, Garver RI Jr (1997) Virology 231:201–209], but their effects in the inner ear have not been documented. We investigated the ability of one HDI, trichostatin A (TSA), to enhance AdV-mediated transgene expression in inner ear tissue. We cultured neonatal rat macular and cochlear explants, and transduced them with an AdV encoding green fluorescent protein (Ad-GFP) under the control of a constitutive promoter for 24 h. In the absence of TSA, GFP expression was limited, and very few hair cells were transduced. TSA did not enhance transduction when applied at the onset of Ad-GFP transduction. However, administration of TSA during or just after Ad-GFP application increased GFP expression in supporting cells approximately fourfold. Moreover, vestibular hair cell transduction was enhanced approximately sixfold, and that of inner hair cells by more than 17-fold. These results suggest that TSA increases AdV-mediated transgene expression in the inner ear, including the successful transduction of hair cells. HDIs, some of which are currently under clinical trials (Sandor et al., 2002), could be useful tools in overcoming current limitations of gene therapy in the inner ear using Ad-GFP. Published by Elsevier Ltd on behalf of IBRO.

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Abbreviations: AdV, adenovirus vector; Ad-GFP, adenovirus vector encoding green fluorescent protein; Ad-LacZ, adenovirus vector encoding β-galactosidase; CAR, coxsackie and adenovirus receptor; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; GFP, green fluorescent protein; HATs, histone acetyltransferases; HCs, hair cells; HDACs, histone deacetylases; HDIs, histone deacetylase inhibitors; PAGE, polyacrylamide gel electrophoresis; PFA, paraformaldehyde; PI, propidium iodide; PVDF, polyvinylidene difluoride; RAR, retinoic acid receptor; RXR, retinoid X receptor; T-PER, tissue protein extraction reagent; TSA, trichostatin A.

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The major cause of sensorineural hearing loss and vestibular disorders is the loss of hair cells (HCs) in the inner ear (Nadol, 1993). This can occur for many reasons, including aging, genetic disorders and exposure to noise or toxins. Moreover, although HC regeneration occurs in birds and fish, the loss of HCs in mammals is currently irreversible. It is thus important to develop therapies that can prevent HC loss or induce HC regeneration in patients.

Potential treatments for inner ear disorders include gene therapy. It has been demonstrated experimentally that gene transfer into the inner ear can induce the expression of protective substances (Kawamoto et al., 2003), correct genetic disorders (Maeda et al., 2007), and induce HC regeneration (Izumikawa et al., 2005). Gene transfer into the inner ear also has the advantage that this site is relatively isolated from other tissue and the spread of gene transfer vectors to other organs is likely to be limited.

Adenovirus vectors (AdVs) have been widely explored for gene therapy because of their ability to mediate transgene expression in many cell types. However, adenoviral toxicity restricts their use for clinical medicine, and they do not efficiently transduce all types of cells. This is particularly true of HCs, which are one of the most important targets for gene therapy of the inner ear. Augmentation of gene expression efficacy from AdVs may be helpful for inner ear gene therapy because it would potentially decrease the quantity of vector required for therapy and thus decrease the potential for toxicity. It may also increase the numbers and types of inner ear cells that can effectively be transduced.

Acetylation of core histones is an important regulator of gene expression in eukaryotic cells, since it reduces the density of nucleosomes and enhances exposure of DNA to the transcriptional machinery of the cell. Histones may be involved in efficient transduction with vector DNA, as well. Viruses produce histone-like proteins and recruit them to their DNA, which can increase transcriptional access (Grove and Saavedra, 2002). A histone deacetylase inhibitor (HDI): trichostatin A (TSA) has been shown to enhance AdV-mediated transgene expression in a number of cell types (Dion et al., 1997), and this property may be helpful in overcoming some limitations of gene therapy using AdVs. However HDIs have not been evaluated for their potential to enhance AdV-mediated gene expression in the inner ear. The present study was designed to assess the effects of TSA on transgene expres-

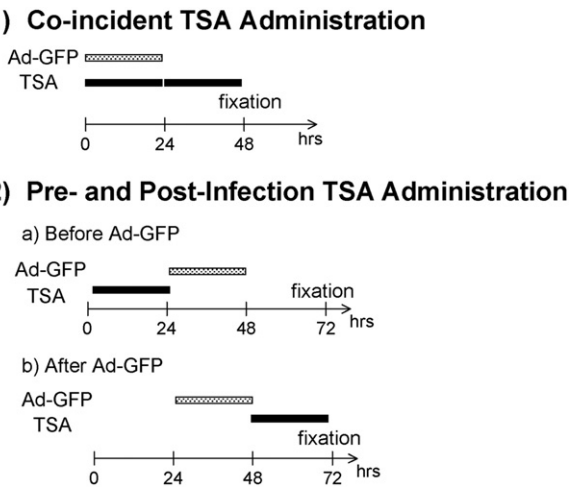

Fig. 1. Experimental protocols used in the present study. TSA was applied during, before or after exposure of the tissue to Ad-GFP.

sion mediated by AdVs in inner ear cells, and to determine whether TSA altered core histone acetylation.

EXPERIMENTAL PROCEDURES

Adenoviral vectors (AdVs)

Replication-incompetent AdVs (E1/E3 deleted, CMV promoter, serotype 5 expressing green fluorescent protein (Ad-GFP) or β-galactosidase (Ad-LacZ)) were used.

Cell culture and treatment

As a positive control for the effects of TSA, the Rat-1 fibroblast cell line was infected with Ad-GFP at 30 PFU/cell. After infection, the cells were cultured in the presence or absence of 500 nM TSA for 48 h. The effect of TSA on GFP expression was evaluated by fluorescence microscopy.

Tissue culture of vestibular maculae and organ of corti

Utricular and saccular maculae and organs of Corti were dissected from Wistar rat pups at postnatal days 3–5 (P3–5). The vestibular explants were then transferred into a 24-well plate and cultured with the HC layer uppermost. The explants were maintained in culture medium composed of Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 30 U/ml penicillin, to which HEPES buffer had been added to a concentration of 25 mM.

Culture wells contained 500 μl of medium and were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The experimental protocol adopted for this study was approved by the Animal Subjects Committee of the San Diego VA Medical Center.

Administration of TSA and Ad-GFP or Ad-LacZ

The explants were incubated in the serum-free medium containing 1×10^7 PFU/ml of Ad-GFP or Ad-LacZ for 24 h. The medium was then replaced with fresh DMEM containing 10% FBS, and TSA (500 nM final concentration) was added to the medium at designated times (Fig. 1).

Measurement of transduction efficiency

Forty-eight hours after transduction with Ad-GFP, the explants were fixed with 4% paraformaldehyde (PFA) for 30 min. Explants were first evaluated as whole-mounts under fluorescence and confocal microscopy. They were then embedded in OCT compound and sectioned at 10 μm on a cryostat. The sections were nuclear-stained with DAPI and immunostained for myosin 7A to distinguish HCs from supporting cells. The percentages of GFP-positive cells were normalized to the number of DAPI-positive cell nuclei. Expression of Ad-LacZ was examined by whole mount X-gal staining followed by sectioning. Explants were fixed with 4% PFA and immersed in X-gal solution. They were then embedded in OCT and sectioned at 10 μm.

Western blotting for detection of histone acetylation

TSA-treated and normal control vestibular maculae were evaluated for acetylation of histones by Western blotting. Twenty vestibular organs from P4 rats were lysed in 400 μl T-PER (Tissue Protein Extraction Reagent). The samples were then spun down at 1000 rpm for 2 min and the supernatant discarded. The tissue was then homogenized. NuPAGE LSD sample buffer and NuPAGE Reducing Agent (Invitrogen, Carlsbad, CA, USA) were mixed with the samples and they were heated to 70 °C for 3 min. The samples were then kept on ice for a few minutes. Thirty microlitres of tissue lysates were separated by SDS-polyacrylamide gel electrophoresis (PAGE) on 7.5% gels and electrotransferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P; Millipore, Bedford, MA, USA). The membranes were blocked with 5% nonfat dried milk in TBS–Tween [50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 0.1% Tween 20] for 60 min at room temperature. The blots were incubated with a primary antibody (mouse monoclonal anti-acetyl-lysine antibody AKL5C1, (sc-32268; Santa Cruz Biotechnology, Santa Cruz, CA USA) that detects N-epsilon-acetylated lysine residues) in blocking buffer overnight at 4 °C and then incubated with horseradish peroxidase-

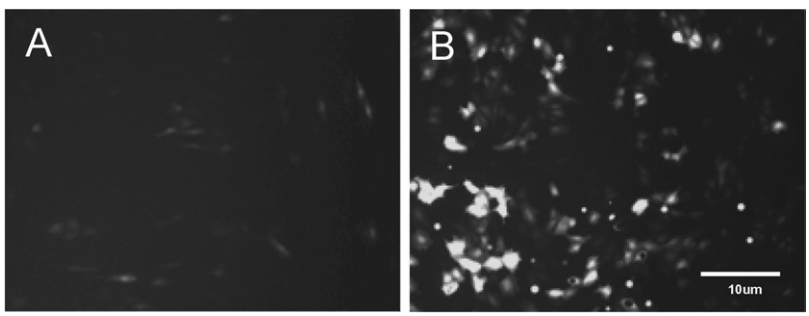

Fig. 2. Effect of TSA on Ad-GFP transduction of rat fibroblasts. (A) Ad-GFP alone: Only a few GFP-positive cells are observed. (B) Ad-GFP+TSA: Many more GFP-positive cells are present and transduction levels are increased, as has been reported previously [4].