Characterization of Adenovirus-Mediated Gene Transfer in Rabbit Flexor Tendons

Vishal Mehta, MD, *Chicago, IL,*
Quan Kang, MD, *Chicago, IL, Chongqing, China,* Jeffrey Luo, MD,
Tong-Chuan He, MD, PhD, Rex C. Haydon, MD, PhD,
Daniel P. Mass, MD, *Chicago, IL*

Purpose: Adenoviral vector–based gene therapy is a promising technique for the delivery of growth factors to tendons. The objective of this study was to determine whether rabbit flexor tendons could be transduced effectively by adenoviral vectors and whether the introduction of adenoviral vectors would cause a notable local inflammatory response.

Methods: Recombinant adenoviruses expressing green fluorescent protein (AdGFP) or BMP-13 (AdBMP-13) were constructed and 3 different viral titers (1×10^7, 1×10^8, and 1×10^9) were tested in this study. The second through fifth tendons of the forepaws and hindpaws of a New Zealand white rabbit were identified surgically and injected with different viral titers of adenoviruses. The fifth tendon was used as a control. The tendons were harvested 12 days after surgery. The retrieved tendons were sectioned to measure transgene expression, as well as for histologic evaluation.

Results: At all tested viral titers an efficient dose-dependent transgene expression was detected in all samples at 12 days after injection. At the highest dose the injection sites were notable for lymphocytic infiltration, suggesting that injected adenoviral vectors can illicit some local inflammatory response. Lymphocytic infiltration was much less apparent, however, in the tendons injected with lower titers of adenoviral vectors. There was no evidence of a massive inflammatory response and/or cell death.

Conclusions: Our findings show that adenovirus-based gene therapy is an efficient means of gene delivery to rabbit flexor tendons. Transduction efficiency of transgenes was dose dependent across the tested titers, although adenovirus-induced inflammation was notable only at the highest titer. This indicates that efficient gene transfer without notable local inflammatory response may be achieved by using the lower titers. Although adenovirus-induced inflammation can be minimized by using lower viral titers, its impact on adhesion formation in the long term remains unknown. (J Hand Surg 2005; 30A:136-141. Copyright © 2005 by the American Society for Surgery of the Hand.)

Key words: Adenoviral vector, bone morphogenetic protein (BMP), flexor tendon, gene therapy, inflammation.

From the Molecular Oncology Laboratory, Department of Surgery, The University of Chicago Medical Center, Chicago, IL; and the Children's Hospital of Chongqing University of Medical Sciences, Chongqing, China.

Received for publication April 29, 2004; accepted in revised form August 16, 2004.

Supported in part by research grants from the Orthopaedic Research and Education Foundation. V.M. was a recipient of the Orthopaedic Research and Education Foundation Resident Research Award. Q.K. was a recipient of an International Postdoctoral Fellowship from the National Institutes of Health (F05 AT002014-01).

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Reprint requests: Daniel P. Mass, MD, Professor of Surgery, Department of Surgery, Section of Orthopaedic Surgery, The University of Chicago Medical Center, 5841 S. Maryland Ave, MC 3079, Chicago, IL 60637.

0363-5023/05/30A01-0021$30.00/0
doi:10.1016/j.jhsa.2004.08.010

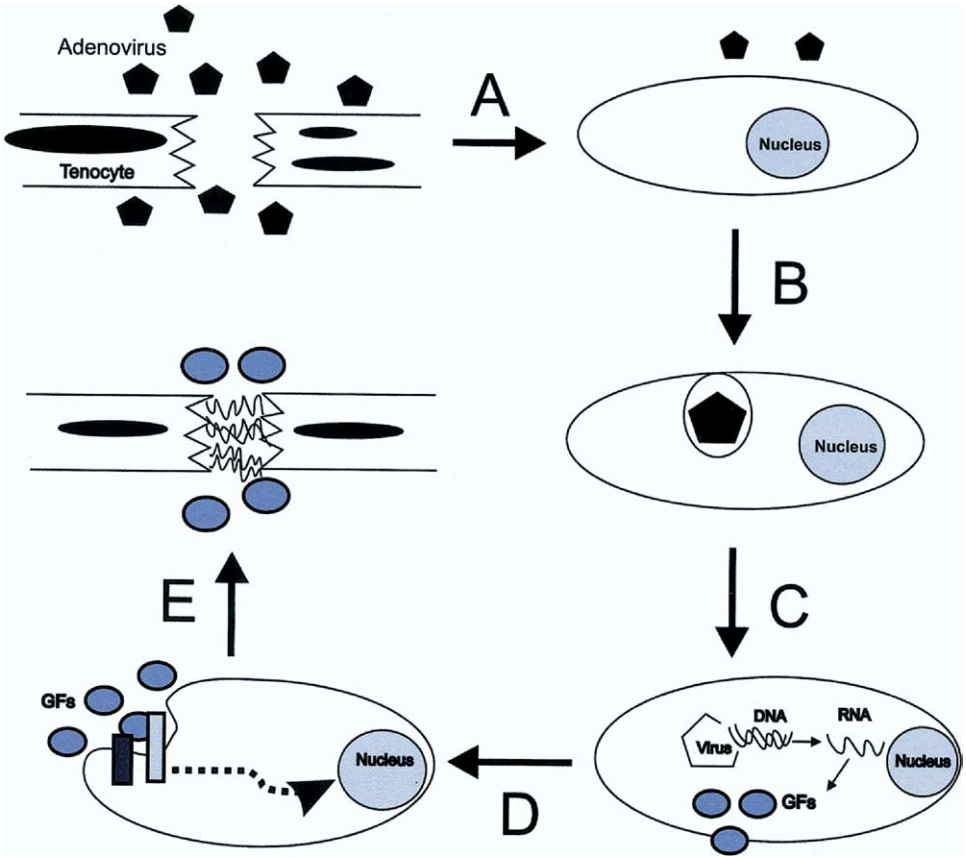

Figure 1. The use of gene therapy in tendon repair. (A) The adenovirus vectors expressing growth factors (such as BMPs) are delivered to the site of tendon laceration. (B) The viruses attach to the cell membrane of the tenocyte and next (C) enter the cytoplasm. (D) The DNAs encoding for the growth factors are transcribed to RNA and then translated to proteins (GFs). (E) The tenocyte then secretes the factors into the extracellular environment where they can bind to receptors on nearby cells and promote tendon healing.

Flexor tendon lacerations represent a common yet challenging problem. Outcomes of surgical treatment often are compromised by rupture or adhesions.[1,2] Attempts to decrease the rupture rate, such as immobilization or bulky suture technique, often increase adhesions.[3] Conversely attempts to decrease adhesions, such as early active range of motion, often lead to tendon rupture.[4,5] Recently biologic augmentation of flexor tendon healing has been explored as a solution to this dilemma. If the biology of flexor tendon healing can be altered to provide a stronger repair, then earlier and more aggressive therapy potentially could be started. This in turn would lead to fewer adhesions with the end result being a more functional finger.

Attempts to influence the biologic milieu in favor of improved tendon healing has attracted a lot of attention,[6–9] particularly with respect to the bone morphogenetic proteins (BMPs).[10,11] The use of gene therapy to deliver BMPs results in sustained expression of a selected transgene and may be superior to recombinant protein with respect to biological activity and longevity of effect (Fig. 1). We previously showed transgene expression of up to 6 weeks in an *in vitro* tenocyte model (unpublished data). Successful *in vivo* transfection of tendons via adenoviral-based gene therapy also has been shown.[12,13] The collective experience with gene therapy in flexor tendons, however, remains limited. Adenoviral delivery of BMP-12 to a chicken flexor tendon *in vivo* has been successful; however, the optimal viral delivery titer remains elusive.[11] The use of higher titers may provide better rates of transfection but it may cause notable inflammation, which in turn causes tendon adhesions.[14–16] Flexor tendons are relatively immunoprivileged sites and therefore may be less prone to adenovirus-induced inflammation. In this study we used an *in vivo* rabbit flexor tendon model to determine the optimal viral titer for efficient gene transfer with minimal local inflammatory response.