Tendon Healing *In Vitro*: bFGF Gene Transfer to Tenocytes by Adeno-Associated Viral Vectors Promotes Expression of Collagen Genes

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Purpose: Adeno-associated virus-mediated gene transfer is promising in the delivery of genes to tendons because this vector stimulates few adverse tissue reactions. Basic fibroblast growth factor (bFGF) promotes collagen production in healing tendons. We transferred the exogenous bFGF gene to proliferating tenocytes by adeno-associated viral (AAV) vectors and investigated its effects on the expression of the collagen genes in an *in vitro* tenocyte model.

Methods: AAV2 vectors harboring the rat bFGF gene were constructed. Tenocytes were obtained from explant cultures of rat intrasynovial tendons and were distributed into 21 culture dishes and 8 wells. Tenocytes in 7 dishes were treated with AAV2 bFGF for 3 hours and then were cultured for 10 days. Tenocytes in 14 dishes (sham vector and nontreatment controls) did not receive the transgene. Efficiency of the gene transfer was evaluated by *in situ* β-galactosidase staining in 8 wells after treatment with AAV2 lacZ. Expression of the target genes was assessed by reverse-transcription polymerase chain reactions with primers specifically amplifying the target genes. Expression of bFGF and type I and III collagen genes was determined by quantitative analysis of the polymerase chain reaction products.

Results: Positive β-galactosidase staining confirmed the effectiveness of AAV2-mediated gene delivery to tenocytes. The level of expression of the bFGF gene was increased significantly after gene transfer. Levels of expression of type I and III collagen genes after transfer of the exogenous bFGF gene were increased significantly compared with those in the cells treated with sham vectors or in nontreatment controls.

Conclusions: Delivery of exogenous bFGF gene to tenocytes can increase significantly the levels of expression of the bFGF and type I and III collagen genes. AAV2 vectors provide a novel method for delivering growth factor genes to tenocytes. These findings warrant future *in vivo* study of the delivery of genes pertinent to tendon healing through AAV2-based gene therapy to enhance repairs of injured flexor tendons. (J Hand Surg 2005;30A:1255–1261. Copyright © 2005 by the American Society for Surgery of the Hand.)

Key words: Flexor tendon healing, growth factors, gene therapy, adeno-associated viral vectors.

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The delivery of exogenous genes to lacerated intrasynovial flexor tendons provides a new approach to enhance the healing process of injured flexor tendons.[1–5] Previously exogenous genes were transferred to the tenocytes through adenoviral or plasmid vectors. Lou et al[1–3] reported transfer of the *Escherichia coli lacZ* gene, a gene encoding focal adhesion kinase, and the bone morphologic protein-12 gene to chicken tendons using adenoviral vectors. Wang et al[4] transferred the platelet-derived growth factor-B (PDGF-B) gene to tenocytes using plasmid vectors. Transfer of the PDGF-B gene has promoted effectively the expression of the type I collagen gene and increased the expression of the PDGF-B gene in tenocytes. Recently Mehta et al[5] reported transfer of green fluorescent protein and bone morphologic protein-13 genes to rabbit tendons and assessed different degrees of local inflammatory changes when varying titers were used. Adenoviral vectors are immunogenic and generally produce adverse tissue reactions when used at high titers,[6,7] and the liposome that aids in gene transfers by plasmid vectors may cause some adverse tissue reactions or damage.[8,9] In recent years adeno-associated viral (AAV) vectors have been the subject of intense research because of their potential as gene transfer vehicles.[10,11] This vector system is nonpathogenic and nontoxic.[10,11] Basic fibroblast growth factor (bFGF) was proven to promote collagen synthesis and tenocyte proliferation in several studies.[12–15] In this study we transferred the exogenous bFGF gene to proliferating tenocytes by AAV2 vectors and investigated its effects on expression of the collagen genes in an *in vitro* model of the tenocytes.

Materials and Methods

Construction of Adeno-Associated Viral 2–Basic Fibroblast Growth Factor Gene Delivery Unit

Adeno-associated viral 2–basic fibroblast growth factor and AAV2-lacZ vectors were used in this study. The AAV2-bFGF vector contains the rat bFGF gene (X07285; Genebank, National Institutes of Health, Bethesda, MD) and has a nuclear localization signal under the regulation of the cytomegalovirus immediate early promoter.[16] A segment containing bFGF gene was cut through EcoRI and XhoI restriction sites of a plasmid that preserved rat bFGF gene. This segment was cloned to another vector (pBluescript II KS vector; Stratagene, La Jolla, CA) at EcoRI and XhoI restriction sites to lengthen the segment because the ideal length of the inserted expression cassette in the AAV2 vector is between 1 and 3 kilo base pairs (kb). Subsequently a 2.7-kb fragment containing the bFGF gene sequences was cut from the vector (pBluescript II KS vector) and was ligated to a multiple cloning site (MCS) of a pAAV2-MCS vector (Stratagene) (Fig. 1) by using a ligation system (LigaFast Rapid DNA Ligation System M8221; Promega Corp., Madison, WI).

An AAV helper plasmid (pAAV-RC, Stratagene) containing subtype 2 AAV *rep* and *cap* genes was used for the production of AAV vectors. Another plasmid (pHelper, Stratagene) containing the E2A, E4, and VA genes of the adenovirus genome, was also used for viral replication. The pAAV2-bFGF and helper plasmids cotransfected human fetal kidney cells (293 cells) by the calcium phosphate coprecipitation method. After 48 hours 293 cells were harvested and lysed through cycles of freezing and thawing, sucrose precipitation, and CsCl density-gradient ultracentrifugation to isolate AAV2-bFGF. The titer of the AAV2 bFGF was determined by real-time polymerase chain reaction (PCR).

Adeno-associated viral 2 lacZ, which harbors the bacterial β-galactosidase (*lacZ*) gene, was prepared similarly. The commercially available pAAV-lacZ plasmid (Stratagene) was used to cotransfect 293 cells to produce AAV2-lacZ.

Culture of Tendon Explants and Tenocytes

Flexor digitorum profundus tendons from 5 adult rats (Sprague-Dawley, Charles River Laboratory, Wilmington, MA) were used. The rats weighed 250 to 300 g each and were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg). Both of their front feet were sterilized and the flexor digitorum profundus tendon equivalents located deeper to the superficialis tendon equivalents were identified in the digits. Under a surgical microscope a 1-cm–long tendon segment was harvested with a surgical blade at the level of the proximal phalanx. The tendon segments were cut into 5-mm long pieces and were placed in 7 culture dishes for explant culture in Dulbecco's modified Eagle medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (GIBCO) and penicillin/streptomycin (100 U/mL). The culture dishes were placed in a humidified 37°C, 5% CO_2 incubator. Culture for approximately 10 to 14 days allowed the migration of sufficient tenocytes out of the explants and the proliferation to approximate monolayer confluence. The explants were re-