Towards Gene Therapy for Uterine Fibroids: Adeno-associated Virus Can Infect Human and Rat Leiomyoma Cells

Ayman Al-Hendy, John Chiorini, and Giovanni Di Pasquale
Department of Obstetrics & Gynecology, University of Texas Medical Branch, Galveston, Texas

Ashbel Smith Building
"Old Red"
Built in 1891

ABSTRACT

Objectives: Uterine fibroids are the most common pelvic tumor, affecting 20% to 25% of premenopausal women. Currently there is no effective and sustained nonsurgical treatment for uterine fibroids. The development of therapeutic agents capable of reversing the tumor growth in leiomyoma without serious side effects is urgently needed. We have recently demonstrated the ability of a mutated dominant-negative estrogen receptor gene delivered via an adenoviral vector to induce apoptosis in leiomyoma cells, as well as ablate preexisting fibroids in vivo. One of the potential limitations of adenoviral-based vectors is short term expression. Adeno-associated virus (AAV) has been shown to mediate higher transfection ability and longer gene expression. In this study we aim to explore the potential application of AAV to support gene therapy of uterine leiomyoma.

Methods: Four different cell lines were used: an ELT3 rat leiomyoma cell line, HM9 telomerase-immortalized human myometrial cell line, LM176 primary human leiomyoma cells, and HM176 primary human myometrial cells. Cells were grown to 50% confluence and then transfected with different virus-to-cell ratios (1×10^5, 2×10^4, or 1×10^3) of DNAase Resistant Particle (DRP) per cell of RSV-green fluorescence protein recombinant AAV2, AAV4, AAV5, AAV6, or BAAV. Four- to 6-days postincubation, the efficiency of viral transduction was assessed with fluorescence microscope.

Results: AAV2 and BAAV were highly efficient in transducing all leiomyoma cell lines tested, with 100% transduction rate achieved at 10^5 DRP/cell. These two vectors were less efficient in transducing normal myometrial cells. AAV4, AAV5, and AAV6 were unsuccessful in transducing either myometrial or leiomyoma cells.

Conclusion: AAV2 and BAAV are promising vectors in the future development of gene therapy protocols for uterine leiomyoma.

INTRODUCTION

Uterine leiomyoma arise from the smooth muscle compartment of the uterus (myometrium) and are the most common gynecologic tumor of premenopausal women, occurring in upwards of 77% of all women. They are a significant cause of pelvic pain, menorrhagia, infertility, and complications associated with pregnancy, and are the leading indication for hysterectomy in reproductive-age women. Leiomyomas are estrogen-dependent tumors. They display an enhanced responsiveness to this steroid hormone and evidence suggests that the enhanced mitogenic effect of estrogen on these tumors is mediated at least in part by cross-talk between estrogen receptor (ER) and several growth factors, for example, insulin-like growth factor I receptor (IGFI-R) signaling pathways. The hormone-dependent phenotype of uterine leiomyoma suggests that interventions targeting the ER signaling pathway may have therapeutic efficacy. In fact, we have recently reported that adenovirus-mediated delivery of a mutated dominant negative estrogen receptor (Ad-ER-DN) inhibited cell proliferation and induced apoptosis in human and rat leiomyoma cell lines. When Ad-ER-DN–treated cells were injected in nude mice, they supported significantly smaller tumor-formation compared with control. Ad-ER-DN also was successful in ablating preexisting leiomyoma tumors in nude mice. One of the potential limitations of adenoviral-based vectors is short term expression. Adeno-associated virus (AAV) vectors have emerged as potentially better alternatives. Adeno-associated viruses are small replication defective parvoviruses whose genomes can be easily manipulated and recombinant particles produced at high titers. Vectors derived from AAVs can direct long-term transgene expression, and elicit a minimal immune response compared to other systems. The cellular tropism of AAV serotypes may be attributed to differences in the viral coats and the differential expression of cellular proteins required for virus transduction. Despite their similarity in size and genomic structure, a number of AAV serotypes have been described to enter and traffic through cells using distinct pathways. AAV serotype 2 (AAV2) transduction is dependent upon heparin sulfate proteoglycan expression, and it can be competitively impeded by soluble heparin. Other proteins involved in AAV2 transduction include FGFR1, integrin aVb5, and a novel 150 KD protein present on the surface of permissive cells. In contrast, AAV4 and AAV5 are insensitive to heparin competition and exhibit distinct cell tropism. Molecular characterization of the cellular factors required for transduction with these isolates demonstrates that both AAV4 and AAV5 require surface expression of a 2–3 sialic acid. While a principle receptor has not been identified for AAV4, additional experiments have identified the PDGFRs as principle receptors for efficient binding and transduction of AAV5. Other primate isolates are less well-characterized but also exhibit unique tropism. In addition, nonprimate AAVs have demonstrated gene transfer ability in distinct cell types when compared with primate isolates. Bovine AAV (BAAV) is a novel AAV vector with unique tropism. It has recently been shown to be efficient on transfecting inner ear cells.

AIM

To assess the ability of different adeno-associated viral vectors to transfect human or rat leiomyoma cell lines

MATERIALS & METHODS

Viral vector construction

The AAV2 beta actin-GFP fusion expression plasmid was constructed by subcloning of the CMV promoter, beta actin-GFP cassette from beta actin-GFP plasmid (Clontech) into the AAV2 RSV-GFP expression plasmid and replacement of the RSV GFP cassette with the CMV beta actin-GFP. The AAV5 beta actin-GFP fusion expression plasmid was produced in the same manner; however, the CMV beta actin-GFP cassette was cloned into the AAV5 RSV-GFP plasmid.

AAV preparation and quantification

Recombinant AAV particles were produced by triple transfection of 293 T cells with AAV helper plasmids expressing the AAV Rep and Cap genes, a vector plasmid containing the reporter gene flanked by either type 2 ITRs (inverted terminal repeats) (AAV2, AAV4) or AAV5 ITRs (AAV5, BAAV), and the Ad helper plasmid pAd12. Recombinant vectors were purified by fractionation with CsCl-gradient centrifugation. DNAase resistant genome copy titers of the vector preparations were determined by quantitative real time PCR using the TaqMan system (Applied Biosystems) with probes specific to the RSV promoter. Viruses in CsCl were dialyzed for 24 h using 0.5 mL Slide-A-Lyzer (Pierce) in 100 mL of serum free medium, with changing of the medium 3 to 4 times.

Cell culture

The ELT3 cell line is a rat leiomyoma cell line that expresses both estrogen receptor (ER) and progesterone receptor (PR) and is a kind gift from Dr. Cheryl Walker (University of Texas MD Anderson Cancer Center, Smithville, Texas). ELT3 was maintained at 37ºC in 5% CO_2/air in DMEM medium, with 10% fetal bovine serum. Human myometrial and leiomyoma tissue were collected according to the policies of the IRB at the University of Texas Medical Branch. Human leiomyoma explants were collected from hysterectomy specimens and used to establish leiomyoma cells. Primary cultured cells were then analyzed for homogeneity with regards to the myoepithelial population using smooth muscle immunohistochemical markers (•-actin, desmin, and vimentin).

Viral infection and histochemistry

Cultured explants of human and rat leiomyoma cells, as well as human myometrial cells, were infected with AAV2, AAV4, AAV5, and BAAV viral vectors using beta actin-GFP as a reporter gene in 200 µL of DMEM F-12 at 37ºC and 5% CO_2 for the duration of the experiment. For immunohistochemistry, cultures were fixed with 4% paraformaldehyde in PBS for 1 h at room temp, permeabilized for 30 min with 0.5% Triton X-100 in PBS, and the actin filaments were counterstained with rhodamine/phalloidin (0.2 U/200 µL, Molecular Probes) for 30 min. Stained explants were removed from the culture dish and mounted using ProLong anti-fade media. Fluorescence images were obtained with a Zeiss LSM 510 confocal microscope using a 100x 1.4 numerical aperture objective. Image acquisition and postacquisition analyses were performed using NIH Image and Adobe Photoshop.

RESULTS

Figure 1. Transfection of rat ELT3 leiomyoma cells with different AAV vectors 10^5 DRP/cells—3 days

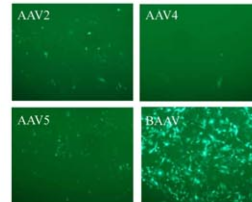


Figure 2. Transfection of human primary myometrial cells with different AAV vectors 10^5 DRP/cells—3 days

Figure 3. Transfection of human primary leiomyoma cells with different AAV vectors 10^5 DRP/cells—3 days

Figure 4. Transfection of human primary myometrial cells with different AAV vectors 10^5 DRP/cells—4 days

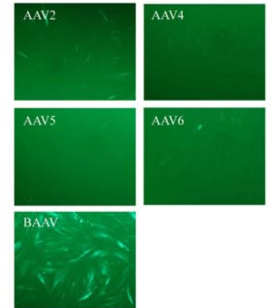
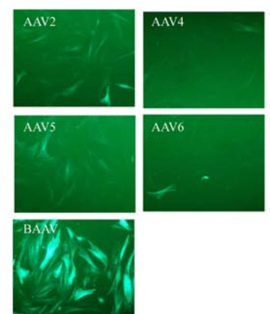


Figure 5. Transfection of human primary leiomyoma cells with different AAV vectors 10^5 DRP/cells—4 days



CONCLUSIONS

BAAV and AAV2 are promising vectors in the future development of gene therapy protocols for uterine leiomyoma.