Uterine Fibroids Gene Therapy: Adenovirus-mediated Herpes Simplex Virus Thymidine Kinase/Ganciclovir Treatment Inhibits Growth of Human and Rat Leiomyoma Cells

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"Old Red"
Built in 1891

ABSTRACT

Introduction: Adenovirus-mediated herpes simplex virus thymidine kinase gene transfer in combination with ganciclovir (Ad-TK/GCV) is one of the major gene therapy strategies to eradicate tumor cells. Leiomyoma is a benign uterine tumor that affects a high percentage of women in their reproductive years. Unfortunately, there is no effective and safe medical treatment available. Leiomyoma is developed as discrete well-defined tumor, easily accessible with imaging techniques, which makes it a good candidate for localized gene therapy approaches.

Objective: To determine the in vitro efficacy of Ad-TK/GCV as a potential gene therapy for leiomyoma using rat ELT-3 leiomyoma cell lines and human leiomyoma cells (LM) as the surrogate system.

Methods: ELT-3 cells and five LMs were infected with different MOI (10–100 PFU/cells) of Ad-TK and treated with different concentrations of GCV (5, 10, or 20 g/mL) for 5 days followed by cell count (DNA content). To test the bystander effect, Ad-TK-transfected ELT-3 cells (100 PFU/cell) or LM cells (10 PFU/cell) were cocultured with corresponding nontransfected cells at different percentages and treated with GCV (10 µg/mL) for 5 days, followed by cell count.

Results: In ELT-3 cells transfected with different MOIs of Ad-TK/GCV (10, 20, 50, or 100 PFU/cell), the cell numbers were reduced by 24%, 42%, 77%, or 87%, respectively compared to the control cells (transfected with Ad-LacZ/GCV). Similarly, in LM cells transfected with Ad-TK/GCV (10, 50, or 100 PFU/cell), cell count was reduced by 31%, 62%, or 82%, respectively, compared to the control. A strong bystander effect was noted in both ELT-3 and LM cells with significant killing ($P = .001$) at a ratio of infected: uninfected cells of only 1:99 and a maximal killing at 1:4.

Conclusion: This study demonstrates the potential efficacy of an Ad-TK/GCV approach in leiomyoma gene therapy.

INTRODUCTION

Uterine Leiomyomas (ULM) are benign proliferations of uterine smooth muscle occurring in one of every three women of reproductive age. The traditional definitive treatment for ULM is hysterectomy. However, hysterectomy is not always an appropriate treatment for women who wish to retain their fertility or have children. Another option is myomectomy. Nevertheless, some women will experience recurrence of ULM after a myomectomy. Likewise, pharmacological treatment such as gonadotropin-releasing hormone (GnRH) agonists are currently limited to short-term use because of serious side effects. Furthermore, GnRH agonist treatment is not appropriate for women who wish to conceive. Due to minimal success with nonsurgical treatment options, it is imperative that other treatment strategies should be assessed as an alternative effective management of leiomyoma. Gene therapy is emerging as promising treatment strategies and several clinical trials are in progress for treatment of several types of tumors. One of the most frequently studied gene therapy strategies is based on transfection with the herpes simplex virus thymidine kinase gene (HSV-TK), followed by ganciclovir administration (GCV), which is an acyclic nucleoside that is normally metabolized at a very low level by mammalian cells. Since ULM is developed as discrete well-defined tumors, easily accessible with imaging techniques, it is a good candidate for localized Ad-TK/GCV gene therapy approaches. Furthermore, higher expression of connexin 43 in ULM compared to the adjacent normal myometrium may suggest an outstanding efficacy of the Ad-TK/GCV gene therapy approach in ULM.

MATERIALS & METHODS

Cell lines

ELT-3 rat leiomyoma cells were grown in DMEM medium supplemented with 10% fetal bovine serum and 1% antibiotic. Human leiomyoma tissue was collected according to the policies of the IRB at the university of Texas Medical branch. Human Leiomyomas explants were collected from hysterectomy specimens and used to establish leiomyoma cell lines (LM). LM cells were grown in DMEM containing 10% FBS and 1% antibiotic.

Recombinant adenovirus

Recombinant replication-deficient adenoviral vector (Ad-TK) expressing the herpes simplex thymidine kinase under transcriptional control of the Rous Sarcoma Virus has been used. The Ad-TK was a gift from Dr. Savio Woo, (Mount Sinai School of Medicine, NY). Adenovirus expressing a marker gene coding for bacterial ß-galactosidase (Ad-LacZ) is a kind gift from Dr Savio Woo (Mount Sinai School of Medicine, NY).

Cell culture infection with Ad-TK

ELT-3 and LM cells lines were maintained as described earlier. For infection with adenovirus vector, the cells were allowed to grow to 60% to 70% confluence and then infected with the Ad-TK or Ad-Lac reporter vector at the desired MOI (100 PFU/cell for ELT-3 cells and 10 PFU/cell for LM cells) for 4 h. Twelve hours after transfection with adenovirus vector, the medium was replaced with medium containing 10 µg GCV/mL. Five days later, the sensitivity to GCV was assessed by measuring cell counts. For the GCV dose-response experiment, cells were grown in 24-well plates and infected with Ad-TK as above. The next day, different concentrations of GCV (5, 10, or 20 µg/mL) were added and then GCV-induced cell deaths were assessed 5 days later using a fluorometric assay, implementing Hoechst 33258 (bisbenzimide).

Detection of a bystander effect in ELT-3 cells and human LM cells infected with Ad-TK

Cells were grown in 100 mm tissue culture plate till 70% to 80% confluence, and then infected with the Ad-TK as above. The next day, these cells, as well as cells from uninfected cultures, were trypsinized and counted. Different ratios of uninfected/infected cells were then mixed together to a total of 20,000 cells and plated in 12-well plates. The medium was removed 24 h later and replaced with a medium with 10 µg GCV/mL. The results were assessed after 5 days. Cells were then counted using the Hoechest DNA assay procedure.

RESULTS

Cell-Killing Efficacy of Gancyclovar in ELT-3 cells transduced with Ad-TK vector

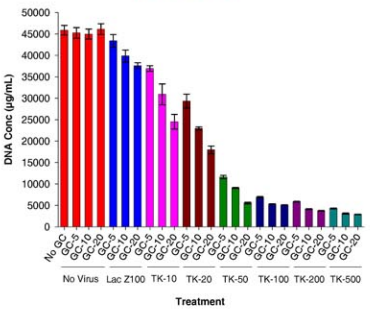

Figure 1. Effect of Ad-TK (MOI of 10–100 PFU/cell) and GCV (5–20 µg/mL) on ELT-3 proliferation. ELT-3 cells were grown in 24-well plates and infected with the Ad-TK or Ad-Lac reporter vector at different MOI for 4 h. Twelve hours after transfection with adenovirus vector, the medium was replaced with medium containing different concentration of GCV. The medium was changed every day with freshly prepared GCV. Five days later, the sensitivity to GCV was assessed by measuring cell count using a fluorometric assay implementing Hoechst 33258 (bisbenzimide).

Cell-killing efficacy of of gancyclovar in LM cells transduced with Ad-TK vector

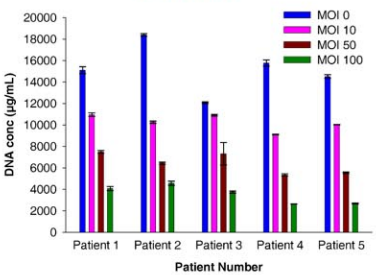

Figure 2. Effect of Ad-TK at different MOI (10–100 PFU/cell) on LM from five different patients. LM cells were grown in 24-well plates and infected with the Ad-TK vector at different MOI for 4 h. Twelve hours after transfection with adenovirus vector, the medium was replaced with fresh medium containing different concentration of GCV. The medium was changed every day with freshly prepared GCV. Five days later, the sensitivity to GCV was assessed by measuring cell count using a fluorometric assay implementing Hoechst 33258 (bisbenzimide).

Bystander effect of Ad-TK/GCV treatment in ELT-3 cells in vitro

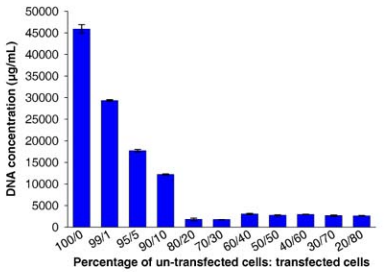

Figure 3. Bystander effect of Ad-TK/GCV on ELT-3 rat leiomyoma cells. ELT-3 cells were transfected with Ad-TK at MOI of 100 PFU/cell. Different ratios of untransfected and transfected cells were culture in 12-well plates and treated with GCV (10 µg/mL). The medium was changed every day with freshly prepared GCV. Five days later, the cell count was measured using a fluorometric assay implementing Hoechst 33258 (bisbenzimide).

Bystander effect of Ad-TK/GCV treatment in LM cells in vitro

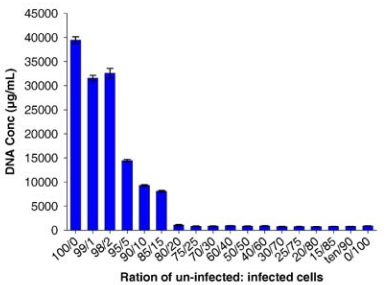

Figure 4. Bystander effect of Ad-TK/GCV on LM cells. LM cells were transfected with Ad-TK at MOI of 10 PFU/cell. Different ratios of untransfected and transfected cells were cultured in 12-well plates and treated with GCV (10 µg/mL). The medium was changed every day with freshly prepared GCV. Five days later, the cell count was measured using a fluorometric assay implementing Hoechst 33258 (bisbenzimide).

CONCLUSION

This study demonstrates the potential efficacy of an Ad-TK/GCV approach in leiomyoma gene therapy