Gene Therapy of Uterine Fibroids: Adenovirus-Mediated Expression of Dominant Negative Estrogen Receptor Inhibits Ere-Reporter Transactivation In Rat and Human Leiomyoma Cells

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Ashbel Smith Building
"Old Red"
Built in 1891

ABSTRACT

Introduction: Uterine leiomyomas (fibroids) are the most common tumors in premenopausal women. Currently, there is no medicinal treatment for this condition and surgery is the mainstay. This constitutes a clinical dilemma, especially in young fibroid patients who desire to preserve their fertility. We have recently demonstrated the ability of a mutated dominant-negative estrogen receptor gene delivered via an adenoviral vector (Ad-ER-DN) to induce apoptosis in leiomyoma cells as well as ablate pre-existing fibroids in vivo.

Objective: To assess the mechanism of Ad-ER-DN-induced apoptosis in leiomyoma cells.

Methods: As experimental models we used primary cultures of human leiomyoma cells (LM155) derived from fibroid tumors removed at hysterectomy as well as rat leiomyoma cells (ELT3). We also used a telomerase-immortalized human myometrial cell line (HM9). Adenovirus carrying two copies of the estrogen responsive element upstream of luciferase reporter (Ad-ERE-Luc) was used at 10 pfu/cell.To investigate the effect of Ad-ER-DN on ERE-reporter transactivation, cells were infected with the therapeutic viral vector for 48 hours. Luciferase expression was measured using standard methods.

Results: In ELT3 rat leiomyoma cells, treatment with Ad-DN-ER at 10, 50, 100, and 200 pfu/cell induced a reduction of ERE-luciferase activity by 30%, 92%, 96%, and 97% respectively ($P < .0001$). A similar trend was observed in HM9, as well as LM155 cell lines. We are currently testing the effect of Ad-ER-DN treatment on the expression of genes involved in apoptosis, angiogenesis, and cell cycling in human leiomyoma cells.

Conclusion: In this work, we demonstrate the ability of dominant negative ER to decrease ERE gene transactivation. This suggests that Ad-ER-DN therapeutic efficacy is at least in part due to perturbing estrogen receptor signaling that is essential for leiomyoma tumor progression.

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. The hormone dependent phenotype of uterine leiomyoma suggests that interventions targeting the ER signaling pathway may have therapeutic efficacy. Proof-of-principal experiments have now established that treatment with antiestrogen medications (eg, tamoxifen and raloxifene) can significantly reduce tumor incidence, size, and proliferative index in the Eker rat model for uterine leiomyoma. Additionally, 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.

AIM

To assess the mechanism of Ad-ER-DN-induced apoptosis in leiomyoma cells.

MATERIALS & METHODS

Cells

As experimental models, we used primary cultures of human leiomyoma cells (LM155) derived from fibroid tumors removed at hysterectomy, as well as rat leiomyoma cells (ELT3). We also used a telomerase-immortalized human myometrial cell line (HM9) at 10 to 200 pfu/cell. To investigate the effect of Ad-ER-DN on ERE-reporter transactivation, cells were infected with the therapeutic viral vector for 48 hours. Luciferase expression was measured using standard methods.

Luciferase reporter assays

Human and rat leiomyoma cell lines were transfected with adenovirus carrying two copies of the estrogen responsive element upstream of luciferase reporter (Ad-ERE-Luc) (kindly provided by Dr. Eun J. Lee, Northwestern University, Chicago, Ill) at 10 plaque forming units (pfu)/cell. After incubation for 5 h at 37°C, the medium was removed, and the cells were incubated in fresh medium with the addition of different concentrations of Ad-ER-DN (1–200 pfu/cell), as well as negative control. After 48 hrs incubation of the transfected cells with different treatments, the cells were harvested. Luciferase activities were determined using luciferase enzyme assay systems with reporter lysis buffer, according to the supplier's protocol (Promega, Madison, Wis). The luciferase activity was normalized against the number of cells/wells calculated by measuring the fluorescence of Hoechst 33258 dye.

Western blot analysis

The following antibodies were used: Bcl2 at a concentration of 1:500; Cyclin D1 at a concentration of 1:300 (Santa Cruz Biotechnology, Inc; Santa Cruz, Calif); ERα at a concentration of 1:200; PR at a concentration of 1:500 (Neo Markers, Inc; Fremont, Calif); COX-2 at a concentration of 1:1000 (Cayman Chemical, Ann Arbor, Mich); and VEGF at a concentration of 1:200 (Santa Cruz Biotechnology).

The ß-actin protein was used as internal loading control, α-actin antibody was used at 1:5000 dilution (Sigma-Aldrich, Saint Louis, Mo). The authenticity of the COX2 band was confirmed using a COX2 blocking peptide, which was mixed with the antibody at a 1:1 (w/w) ratio (Cayman Chemical). Membranes were developed using HRP-conjugated sheep IgG with ECL Western Blotting Detecting Reagents (Amersham Biosciences, UK). The intensity of each band was determined using a scanning densitometer (Epson 4870, Epson America, Long Beach, Calif).

RESULTS

Ad-ER-DN treatment decreases ERE-Luc reporter transactivation in rat leiomyoma ELT3 cells

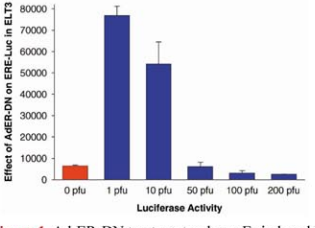

Figure 1. Ad-ER-DN treatment reduces E_2-induced ERE-reporter transactivation in rat leiomyoma ELT3 cell line.

The ELT3 cell line was first transfected with Ad-ERE-tk-Luciferase reporter. Cells were then transfected with various doses (PFU/cell) of Ad-ER-DN and analyzed 24 h later. The difference between high and low viral doses was statistically significant ($P = .0001$). The results are shown at an estrogen concentration of 1 μM, and similar results were obtained at 100 nM, 10nM, 1 nM, and 100 pM. Each value is mean ± SEM of triplicate wells in two independent experiments.

Ad-ER-DN treatment decreases ERE-Luc reporter transactivation in human immortalized HM9 myometrial cells

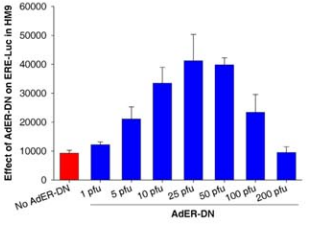

Figure 2. Ad-ER-DN treatment reduces E_2-induced ERE-reporter transactivation in immortalized human myometrial HM9 cell line.

The HM9 cell line was first transfected with an adenovirus expressing wild-type human ER• cDNA, and subsequently was transfected with Ad-ERE-tk-Luciferase reporter. Cells were then transfected with various doses (PFU/cell) of Ad-ER-DN and analyzed 24 h later. The difference between high and low viral doses was statistically significant ($P = .0001$). The results are shown at an estrogen concentration of 1 μM. Each value is mean ± SEM of triplicate wells in two independent experiments.

Ad-ER-DN treatment modulates the expression of several estrogen-regulated genes in human primary leiomyoma LM155 cells

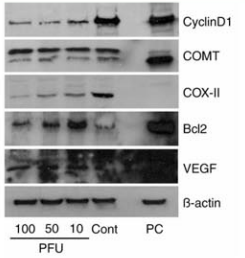

Figure 3. Effect of Ad-ER-DN treatment on cell cycle (cyclinD1, COXII), apoptosis (Bcl2), angiogenesis (VEGF), and estrogen metabolism (COMT) protein expression in primary human leiomyoma LM 155 cells. ß-actin is the internal loading control. PC is the positive control, pfu: plaque forming unit.

CONCLUSIONS

- Adenovirus-mediated delivery of dominant negative ER decreases ERE gene transactivation in leiomyoma cells.
- Several estrogen-dependent genes' expression (CyclinD1, COX-II, VEGF, Bcl2, COMT) is modulated by Ad-ER-DN treatment.
- Ad-ER-DN therapeutic efficacy is at least in part due to perturbing estrogen receptor signaling that is essential for leiomyoma tumor progression.