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Memy I: a novel murine model for uterine leiomyoma using adenovirus-enhanced human fibroid explants in severe combined immune deficiency mice

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Abstract

OBJECTIVE—This study was undertaken to develop a representative murine model for human leiomyoma.

STUDY DESIGN—Human fibroid tumor tissues were cut into small pieces and treated with medium alone, adenoviral- β -galactosidase, adenoviral-vascular endothelial growth factor-A, adenoviral-cyclooxygenase-2, or both adenoviral-vascular endothelial growth factor-A and adenoviral- cyclooxygenase-2. Tissue pieces were inserted subcutaneously in the flank of each severe combined immunodeficient mouse. The developed lesion was measured twice per week. Xenograft tissues were harvested after 30 days and analyzed.

RESULTS—Tissue pieces transfected with both adenoviral-cyclooxygenase-2 and adenoviral-vascular endothelial growth factor-A continued to grow up to 30 days postimplantation. The number of proliferating and apoptotic cells, as well as the expression of smooth muscle actin, desmin, vimentin, estrogen receptors, and progesterone receptors was similar between retrieved grafts from that group and the original patient tissue. Furthermore, hematoxylin and eosin and Masson's Trichrome stains confirmed this similarity.

CONCLUSION—Human uterine leiomyoma xenografts, pretreated with both adenoviral-cyclooxygenase-2 and adenoviral-vascular endothelial growth factor-A and implanted subcutaneously in severe combined immunodeficient mice, represent a novel model for human uterine leiomyoma.

Keywords

cyclooxygenase-2; leiomyoma models; severe combined immunodeficient mice; uterine leiomyoma; vascular endothelial growth factor-A

Uterine leiomyomas, commonly referred to as fibroids, are benign tumors that arise in the myometrial compartment of the uterus.¹ Leiomyomas are well-differentiated smooth muscle tumors with a relatively low mitotic index¹; tumors can become quite large and are usually multiple. Uterine leiomyomas are the most common gynecologic neoplasm, occurring in more than 70% of reproductive-age women.¹ When symptomatic, leiomyomas are associated with infertility, menorrhagia, and spontaneous abortion. They are the leading indication for hysterectomy in premenopausal women.¹ The high frequency of these tumors and lack of satisfactory nonsurgical and fertility-preserving treatments make uterine leiomyomas a significant reproductive health concern for women.¹ The underlying causes of uterine leiomyomas are poorly understood, as a result, in part, of the absence of a good model system with which to study these tumors. The only available animal model is the Eker rat in which both leiomyosarcomas and leiomyomas are observed.² Female Eker rats have uterine leiomyomas develop by 12 to 16 months of age with a frequency of about 65%.²

The predisposing genetic alteration in these animals is an insertion of an endogenous retrovirus between exons 30 and 31 of the *Tsc-2* gene, which inactivates the encoded tumor suppressor protein. This leads to the development of tumors in several different organs, such as the spleen, kidneys, lungs, and uterus.² Although the Eker rat has been valuable as an animal model for uterine leiomyoma, there are limitations regarding difficulty in breeding, low penetrance, high cost, and sarcoma-like histology that hinder its use as a versatile model of human leiomyoma. The rat Ehrlich tumor cells (ELT), cells established from the uterine lesions in the Eker rat, have been established with success.² These cells form leiomyoma-like lesions when implanted subcutaneously in nude mice.² However, the lesions developed in such animals have the same limitations of the original Eker model.²

Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) are the rate-limiting enzymes involved in the conversion of arachidonic acid to prostanoids.³ Although COX-1 is thought to work as a housekeeping gene, COX-2 is inducible and has various cellular functions in addition to its well-known role in inflammatory reactions.³ COX-2 has been linked to tumorigenesis and angiogenesis.³ The expression of COX-2 has been described in epithelial ovarian carcinomas, borderline-malignant ovarian tumors, and many colorectal carcinomas.⁴ Moreover, nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to reduce mortality from colorectal cancer by inhibition of COX-2 activity and tumor angiogenesis.⁴ In breast fibroblasts, COX-2 stimulates P450 aromatase expression,⁵ which enhances E2 synthesis and, in turn, increases the growth rate of these cells. Several reports have suggested that COX-2 overexpression leads to proliferative and angiogenic cellular effects via prostaglandin (PG)-dependant or PG-independent actions.⁶

The vascular endothelial growth factor (VEGF) family includes 7 secreted forms—VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor (PlGF), and VEGF-F. VEGF-A is commonly referred to as VEGF and is the most potent.⁷ VEGF is critical for the initiation and maintenance of the angiogenic response, a major process by which new blood vessels are formed in adults.⁸ It has been documented that angiogenesis plays an important role in the regulation of tumor growth.⁸ It has been shown that VEGF-enhanced

angiogenesis is also associated with an increase in vascular permeability, which results in an increase in the amount of growth factors and nutrients delivered to tumor cells.⁹

Severe combined immunodeficient (SCID) mice possess a combined congenital deficiency in T- and B-lymphocyte function and have been shown to host various heterotransplants successfully, as they cannot elicit humoral and cellular immune responses and, therefore, cannot reject transplanted nonautologous tissues.¹⁰ Human tissues serially passed in these mice were shown to retain their morphologic and biochemical characteristics.¹⁰

The aim of our study was to develop a reliable in vivo model of human uterine leiomyoma by using SCID mice implanted with human leiomyoma explants that were enhanced by transfection with an adenovirus carrying COX-2 and/or VEGF-A.

Materials and Methods

Recombinant adenovirus

Adenoviral vectors, carrying the COX-2 gene under the cytomegalovirus promoter (Ad-COX-2), were a generous gift from Dr Chuan-Ming Hao (Vanderbilt University School of Medicine, Nashville, TN). The Ad-VEGF-A, an adenoviral vector carrying the VEGF-A gene under the cytomegalovirus promoter, was purchased from Vector Biolabs (Philadelphia, PA). The adenovirus expressing a marker gene coding for bacterial β -galactosidase (Ad-LacZ) was a kind gift from Dr Savio Woo (Mount Sinai School of Medicine, New York, NY) and was used as a control for viral transfection. Large-scale production of adenovirus vectors was performed as we have described previously¹¹ with a typical batch yield of 2×10^{10} plaque-forming units (PFU)/mL.

Animals

Forty 2-month-old female SCID mice (SCID/SCID CB17) were purchased from Harlan Sprague Dawley (Indianapolis, IN). Serum estrogen levels were maintained at the highest level by subcutaneous implantation of 1.7 mg, 60-day sustained-release 17 β -estradiol pellets (Innovative Research of America, Sarasota, FL). The pellets were implanted 4 days before the tissue transplantation to allow steady-state tissue estrogen distribution. Animals were kept in microisolator cages and housed separately in a barrier facility with a well-controlled pathogen-free environment, with monitored ambient temperature and regulated cycles of light and darkness, and were fed ad libitum. All experiments involving laboratory animals were performed in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee of the University of Texas Medical Branch (UTMB), Galveston, TX.

Tissue harvest, adenovirus transfection, and implantation

Human leiomyoma tumor tissues were collected from a 44-year-old, gravida 3, para 3 (G3P3) African American female patient at the time of hysterectomy (due to multiple uterine fibroid lesions). The patient had not used any hormonal treatment for at least 3 months before surgery. The fibroid tissues used in this study were from a single, large, and intramural fundal location. We used tissue samples from the same lesion for all animals to

avoid the introduction of variability because of differences in tumor viability, vascularity, cellularity, or other inherent fibroid features. The work was performed in accordance with National Institutes of Health guidelines on the use of human subjects and was approved by the Institutional Review Board of UTMB. Leiomyoma diagnosis was confirmed by the surgeon, based on gross morphologic assessment of the tumor, and by a pathologist on subsequent histologic analysis. Fibroid tissues were harvested aseptically, immediately submerged in tissue culture media, placed on ice, and transferred immediately to the laboratory. Tissue samples were cut into wedge-shaped pieces 3 mm in length and 2 mm in thickness at the broadest end. Wedges were randomly divided into 5 groups based on specific adenovirus transfection as shown in the Table.

The number of cells per tumor was determined using a general rule of 3×10^8 cell/cm³ for leiomyoma tumors.¹² The calculated virus volume was added to the transfection media containing 1% Penstrep and 2% fetal bovine serum. The viral transfection was performed for 5 hours, fresh medium was added, and incubation continued for 12 hours.

For transplantation, 40 SCID mice were divided equally into 5 groups and were then anesthetized with halothane. Small slits (3 mm) were made in the skin of the right flank of each animal, and 2 wedges of tissue were dipped in Matrigel and inserted subcutaneously, and the wounds were then closed with tissue glue. Mice were closely monitored after tissue implantation and the developed lesions were measured twice per week by using an electronic caliper. The tumor volume was determined from 3 diameters by using the ellipsoid formula: volume = $R1 \times R2 \times R3 \times 0.52$.

Tissue evaluation

At 30 days postimplantation, all mice were killed and the experiment was concluded. Xenograft tissues were harvested and examined for gross evidence of vascularization and tissue morphology. The tissues were formalin-fixed, paraffin-embedded, and subjected to hematoxylin and eosin (H&E) histologic evaluation. The histologic sections were graded semiquantitatively for cell density (cellularity), the absence or presence of mitosis per 10 high-power fields, and the degree of collagen changes.

Proliferation was assessed by staining for the expression of the Ki67 protein by using MIB-1 monoclonal antibodies, 1:200 (Dako North America, Inc, Via Real Carpinteria, CA). Apoptosis was evaluated with the ApopTag Kit (Intergen, Purchase, NY) according to the manufacturer's instructions. The degree of apoptosis or proliferation was scored by counting the tunnel or Ki67 positive nuclei (brown), respectively, against the total number of the cells in 4 random high-power fields (total number: 2000 cells) for each tissue section (representing 1 animal). The percentage expressions were calculated, and 3 animals per group were included in these calculations. Data are presented as mean $\pm$ standard error of the mean (SEM) for all tested animals per group.

Expression of the estrogen receptor(ER) and the progesterone receptor (PR) was evaluated with an anti-ER alpha antibody, 1:25 (Santa Cruz Biotech, Santa Cruz, CA) and aPRantibody 1:50(NeoMarkers, Fremont, CA). We also used immunohistochemistry to evaluate typical histologic features of leiomyoma, including antismooth muscle actin

(undiluted; Gene Tex, Inc, San Antonio, TX), antismooth muscle Desmin 1:30 (Santa Cruz Biotech), and antismooth muscle vimentin 1:30 (Santa Cruz Biotech). The collagen content of the lesions was evaluated using Masson's Trichrome staining.

Statistical analysis

The data are presented as mean $\pm$ SEM for all 8 animals from each of the 5 groups in the tumor size experiment and for 3 animals from each group for all other parameters. Group differences were analyzed by using a 1-way analysis of variance (ANOVA), followed by a Tukey test as a post-ANOVA multiple comparison test; $P < .05$ was considered statistically significant.

Results

Uterine leiomyoma xenografts transfected with both Ad-VEGF-A and Ad-COX-2 continued to increase in size in vivo

Human fibroid tissues were obtained from patients undergoing hysterectomy secondary to benign uterine leiomyomas. Tissues were cut into small wedges and then categorized into 5 groups according to specific viral transfection. Group 5, transfected with both Ad- VEGF-A and Ad-COX-2, remained engrafted and continued to grow for 1 full month after transplantation. All other groups showed abrupt and continuous time-dependant decreases in size, as shown in Figure 1. The lesions in these groups (1–4) were barely visible by 2 weeks' postimplantation and had practically vanished 4 weeks after implantation. At the time of harvest from the mouse host, 2–3 clearly visible subcutaneous vessels were connected to the xenograft tissues in group 5.

COX-2/VEGF-A-enhanced xenografts retained typical histologic characteristics of leiomyoma in vivo

We evaluated the ability of human uterine leiomyoma xenografts to retain various histologic and molecular characteristics after subcutaneous implantation in mice. We also assessed the effect of pretreatment with Ad-VEGF-A and Ad- COX-2, separately or in combination, on this process. As shown in Figure 2, the histopathologic evaluation of the xenograft recovered from group 5 mice, pretreated with a combination of Ad- COX-2 and Ad-VEGF-A, exhibited similar histologic features to that of the freshly processed patient tissue specimens. TheH&Estaining analysis showed that both freshly processed patient specimens and recovered grafts from group 5 mice exhibited high cellular leiomyoma nodules composed of well-differentiated leiomyocytes that had formed bundles coursing in several directions (Figure 2, **D**). The leiomyoma cells had an abundant eosinophilic cytoplasm with oval or elongated nuclei that showed a dense nuclear chromatin pattern. Mitotic figures were rare, which is consistent with benign leiomyoma histopathology. A similar staining pattern was obtained from the original matched preimplantation control tissue (Figure 2, **A**). From the residual graft in Ad-LacZ–treated (group 2) and untreated grafts (group 1), we retrieved a small amount of tissue that we processed and used for various histologic tests. When stained with H&E, these small grafts demonstrated minimal cellularity and only some residual extracellular matrix (Figure 2, **B, C**). This is consistent with the sizing data in Figure 1 and suggests that these grafts did not vascularize and engraft successfully.

Proliferation and apoptosis indices in engrafted tissues

Immunohistochemical analysis with anti-Ki67 antibodies and ApopTag analysis revealed that the numbers of proliferating and apoptotic cells were similar among the original patient leiomyoma tissue and matched 1-month xenografts from group 5 (pretreated with Ad-COX-2 and Ad-VEGF-A) (Figure 3, **A, B**). In general, proliferation was slightly higher in the COX-2 and VEGF-A xenograft- treated group (11%) compared with the original tissue (8%) with no significant difference (Figure 3, **A, C**).

Steroid receptors and smooth muscle markers are expressed by engrafted tissues

Immunodetectable ER and PR protein levels were comparable between control leiomyoma tissues and matched Ad-COX-2/Ad-VEGF-A-enhanced xenografts, with respect to both the staining intensity and the staining pattern (Figure 4, **A,B**). These tissues were characterized further regarding the expression of several smooth muscle markers, including basal cell-specific, smooth muscle actin, desmin, and vimentin. The expression of alpha smooth muscle actin, smooth muscle desmin, and smooth muscle vimentin were all comparable among xenografts recovered from group 5 (enhanced with Ad- VEGF-A and Ad-COX-2) and matched original fresh leiomyoma tissue, when tested with human-specific antibodies (Figure 5, **A–C**). The cytoplasm of the leiomyoma cells was strongly labeled for smooth muscle alpha actin (Figure 5,**A**).A strong reaction to vimentin was observed in the connective tissue (Figure 5, **B**).

Collagen production by engrafted human leiomyoma tissue

As shown in Figure 6, Masson's Trichrome staining showed that the extracellular collagen content was largely similar in group 5 and matched fresh tissue. The human leiomyoma cells in group 5 presented a typical staining pattern and showed tumor cell bundles surrounded by moderate amounts of well-vascularized connective tissue.

Both the Ad-LacZ-treated and the media-treated control groups showed a dramatic loss of leiomyoma architecture manifested by a marked decreased in cellularity and the appearance of collagen degradation (Figure 6).

Comment

The goal of this study was to generate a reliable human uterine leiomyoma model in mice by using human tissue. This model is useful for studying the biology of uterine fibroids and for testing novel experimental therapies. Our study suggests that human leiomyoma tissue could be made to engraft and survive for at least 1 month as subcutaneous xenografts in SCID mice after enhancement with both Ad-COX-2 and Ad-VEGF-A. The implanted tissues successfully maintained their volume throughout the experiment and showed modest growth. The engrafted human leiomyoma tissues clearly retained their key features regarding the tissue morphology, indices of apoptosis and proliferation, as well as the expression of various uterine leiomyoma key markers, eg, ER, PR, alpha smooth muscle actin, desmin, and vimentin. Interestingly, all parameters were similar, if not identical, when compared with the original freshly processed matched control leiomyoma lesions. The efficiency with which our short-term xenografts remain viable was rather impressive. We

used the tissue cutting and processing procedure that was described by Presnell et al,¹³ who hypothesized that local nutrients are able to enter the newly transplanted tissue wedges through simple diffusion at the tapered end, which facilitates survival until the development of neovascular connections with the host.

We selected the SCID mouse model because it is devoid of specific immune responses that facilitate the engraftment of human tissues and prolong the Admediated gene expression.¹⁰ It was obvious in the H&E-stained sections that the xenografts are not necrotic or fibrotic, and vascularization was evident because of the presence of small vessels and red blood cells (Figure 2). Thus, our results confirmed that pretreatment with both Ad-COX-2 and Ad-VEGF-A provided a means of satisfactory tissue homeostasis with sustained proliferation and minimal apoptosis that were similar to the results of the matched control leiomyoma tissue.

Ad-COX-2 and Ad-VEGF-A were chosen for the enhancement of leiomyoma tissue because of ample evidence derived from previous reports that link these 2 genes to tumorigenesis, as well as maintenance of tumor cell growth. Selective COX-2 inhibitors have been shown to have strong chemopreventative actions against colon carcinogenesis in rats,¹³ whereas higher COX-2 levels have been observed in many human cancers, such as cancer of the lungs, breasts, colon, prostate, head, and neck^{3,14–16} compared with their normal tissue. COX-2 was also found to sustain tumor growth by enhancing angiogenesis and conferring resistance to apoptosis through stimulation of Bcl2 expression, decreasing both E-cadherin and transforming growth factor B2 receptor expression,¹⁷ as well as attenuating TNF-related apoptosis inducing ligand (TRAIL)-induced apoptosis and transcriptional repression of death receptor-5.¹⁸ Conversely, treatment of cancer cells with COX-2 inhibitors induced caspase-3 activity, which led to cancer cell apoptosis.¹⁹ In addition, COX-2 enhanced cell proliferation by increasing the synthesis of PGE2 and thromboxane.⁶

It is widely accepted that the establishment of nutrient blood vessels is fundamental to the growth of tumors.⁸ Uterine leiomyomas have been associated with irregular vascular networks and angiogenic growth factors, including VEGF.⁹ We anticipated that the overexpression of VEGF-A via adenovirus transfection could enhance the tumorigenicity of human leiomyoma tissue in SCID mice. VEGF is the most potent angiogenic factor known and may enhance the growth of fibroids by increasing their nutrient supply.⁹ VEGF may also have an indirect effect by inducing the proliferation of endothelial cells, which produce a number of growth factors.⁹ VEGF can also release the angiogenic factor, bFGF, from its storage in heparin sulfates of the extracellular matrix, which has a synergistic effect on angiogenesis.²⁰

The overexpression of VEGF has been shown to confer a growth advantage for many cells without leading to malignant transformation, eg, Chinese hamster ovary (CHO) cells.²¹ In another report, VEGF overexpression in low tumorigenic U251MG(glioma) cells promoted tumorigenicity and resulted in the formation of large hemorrhagic subcutaneous tumors and solid tumors in the brain.²²

Many reports indicate compelling evidence for a positive relationship between COX-2 and VEGF. COX-2 induces the production of VEGF through both the PGE₂/PGE₂ membrane receptor and the thromboxane A₂-mediated effect and, which promote neovascularization and tumor angiogenesis.²³ This induction is blocked by the COX-2-specific inhibitor, NS-398.²⁴ VEGF increases COX-2 gene expression at both the messenger RNA and protein levels in cultured human vascular endothelial cells through the p38 MAPK and JNK signal pathways.²⁵

Furthermore, COX-2 is significantly related to VEGF production in glioma and colon cancer.^{14,26} In addition, VEGF also increases thromboxane A₂ synthesis.²⁷ Our data agreed with these reports and demonstrated that only xenografts enhanced with both Ad-COX-2 and Ad- VEGF exhibited a time-dependent increase in size subcutaneously in SCID mice (Figure 1) and retained all the key features of leiomyoma (Figures 2–6). This suggests a synergistic effect between the COX-2 and VEGF signaling pathway in uterine leiomyoma tissue.

Some advantages of our novel model, developed as an advancement tool for uterine leiomyoma understanding and control, include the potential establishment of efficient in vivo xenograft cultures from human leiomyoma, low cost, and a preserved benign leiomyoma phenotype. Furthermore, it enables the possibility of generating mice that bear multiple xenografts derived from different patient samples, so that experiments can be conducted in a large scale of patient tissues by using only a few mice. Likewise, mice that contain multiple xenografts from the same specimen can be generated, which allows grafts to be sequentially harvested for analysis at multiple timepoints during an experiment.

In conclusion, human uterine leiomyoma xenografts were successfully engrafted in SCID mice for 1 month after preimplantation treatment with both Ad-COX-2 and Ad-VEGF-A. The xenografts were well-vascularized and maintained fibroid tissue architecture, as well as the expression of key markers. This model represents a useful tool for in vivo study of human uterine fibroids.

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Figure 1. Human uterine leiomyoma explants in SCID mice

Fibroid explants placed under the skin of SCID mice continued to grow for 30 days in the group transfected with both the adenovirus carrying cyclooxygenase-2 (Ad-COX-2) and the adenovirus carrying the vascular endothelial growth factor (Ad-VEGF). All other groups showed abrupt resorption of the implanted fibroid tissues. The tumor size was measured twice per week with calipers without killing the animals exploiting the accessible subcutaneous location of the tumor lesions. Tumor size was calculated as a percentage of day 0 (preimplantation) volume. Each timepoint is represented as mean $\pm$ standard error of

the mean obtained for all 8 animals in each of the 5 groups. The caliper measurement was stopped when the lesion size became immeasurable in groups that show abrupt resorption, such as the VEGF group, after 3 weeks. *, **, †, and # indicate significant differences from the control, the adenovirus carrying the beta galactosidase gene (Ad-LacZ), Ad-VEGF, or Ad-COX-2 using the Tukey test as a postanalysis of variance test ($P < .05$). At the point representing day 27, a 2-tailed Student *t* test was used to compare data obtained from Ad-COX-2 group vs the combination group ($P < .05$).

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Figure 2. Histologic evaluation of retrieved engrafted human uterine leiomyoma tissues after 1 month postimplantation

Representative hematoxylin and eosin-stained histologic sections from matched preimplantation **A**, freshly processed human uterine leiomyoma vs **B**, implanted uterine leiomyoma retrieved after 1 month in vivo in SCID mice. Explants were pretreated with medium, **C**, the adenovirus carrying the beta galactosidase gene, or **D**, both the adenovirus carrying the vascular endothelial growth factor and the adenovirus carrying cyclooxygenase-2. The tumor cellularity of the fresh human uterine leiomyoma and the explants from mice transfected with both the adenovirus carrying cyclooxygenase-2 and the adenovirus carrying the vascular endothelial growth factor is comparable. Original magnification was $\times 40$.

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Figure 3. Apoptosis and proliferation indices in engrafted tissues

The distribution of **A**, proliferative cells, as identified by antibodies to Ki67 (dark-brown nuclear staining), and **B**, apoptotic cells (apoptag-labeled cells with dark-brown nuclei), were evaluated in the original leiomyoma tissues and the matched 1-month xenograft tissues. There was no significant difference in the percentage of proliferative or apoptotic cells detected in the xenograft retrieved from group 5 (combination) and the **C**, original leiomyoma tissue. The degree of apoptosis or proliferation was scored by counting the tunnel or Ki67 positive nuclei (brown), respectively, against the total number of the cells in

4 random high-power fields (total number 2000 cells) for each tissue section (representing 1 animal). The percentage expressions were calculated for 3 animals per group and were included in these calculations. Data are presented as mean $\pm$ standard error of the mean for all tested animals per group. Original magnification, $\times 40$. *, †, and ‡ indicate significant differences from original, control, or the adenovirus carrying the beta galactosidase gene by using the Tukey test as a postanalysis of variance test ($P < .05$).

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Figure 4. Distribution of the estrogen receptor and progesterone receptor in engrafted human leiomyoma tissues

Immunohistochemistry with antibody against the **A**, estrogen receptor and **B**, progesterone receptor of histologic sections from matched control human uterine leiomyoma vs implanted uterine leiomyoma in SCID mice transfected with the adenovirus carrying the beta galactosidase gene, the adenovirus carrying the vascular endothelial growth factor, and the adenovirus carrying cyclooxygenase-2, or medium. Note that the amount **A**, estrogen receptor or **B**, progesterone receptor, as well as the staining pattern of sections obtained from the xenografts transfected with both the adenovirus carrying cyclooxygenase-2 and the adenovirus carrying the vascular endothelial growth factor, were comparable with the original human uterine leiomyoma sections. Tumors of mice treated with either the adenovirus carrying the beta galactosidase gene or medium alone showed a markedly decreased amount of positive smooth muscle cells and an increase in fibrosis. Original magnification, $\times 40$.

Hassan. Memy I: a novel murine model for uterine leiomyoma. *Am J Obstet Gynecol* 2008.

Figure 5. Expression of uterine leiomyoma smooth muscle markers in engrafted tissues

Freshly processed leiomyoma tissues and 1-month implanted xenografts were characterized for the expression of alpha **A**, smooth muscle actin, **B**, smooth muscle desmin, and **C**, smooth muscle vimentin. Note that the abundance of smooth muscle cells in the original human uterine leiomyoma and the matched tumor from the group pretreated with both the adenovirus carrying cyclooxygenase-2 and the adenovirus carrying the vascular endothelial growth factor were comparable. The section from tumors transfected with either the adenovirus carrying the beta galactosidase gene or medium alone shows a marked decrease in the expression of smooth muscle cell markers and an increase in fibrosis. Original magnification, $\times 40$

Hassan. Memy I: a novel murine model for uterine leiomyoma. *Am J Obstet Gynecol* 2008.

Figure 6. Masson's Trichrome staining for collagen secretion

The amount of extracellular collagen in **A**, preimplantation human uterine leiomyoma tissue and matched tumor tissue transfected with the adenovirus carrying cyclooxygenase-2 and the adenovirus carrying the **D**, vascular endothelial growth factor is comparable. Note the increase in fibrosis and scarcity of collagen in the tumors pretreated with either the adenovirus carrying the **C**, beta galactosidase gene or **B**, medium alone. Original magnification, $\times 40$.

Hassan. Memy I: a novel murine model for uterine leiomyoma. *Am J Obstet Gynecol* 2008.

TABLE

Human leiomyoma explant groups according to preimplantation adenovirus transfection

Group ID	Treatment	PFU/cell
1	Medium alone (negative control)	0
2	Ad-LacZ (positive control for Ad transfection)	50
3	Ad-VEGF-A	50
4	Ad-COX-2	50
5	Ad-VEGF-A and Ad-COX-2	25 each virus

Ad-COX-2, adenovirus expressing cyclooxygenase 2; *Ad-LacZ*, adenovirus expressing a marker gene coding for bacterial β -galactosidase; *Ad-VEGF-A*, adenovirus expressing vascular endothelial growth factor A; *PFU*, plaque forming units.

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