A novel mouse model that closely mimics human uterine leiomyomas

Michael Drosch, M.Sc.,[a,b] Jörn Bullerdiek, Prof. Dr.,[a] Thomas M. Zollner, M.D.,[b] Florian Prinz, M.D., Ph.D.,[b] Markus Koch, Ph.D.,[b] and Nicole Schmidt, Ph.D.[b]

[a] Center of Human Genetics, University of Bremen, Bremen; and [b] Global Drug Discovery–TRG Oncology/Gynecological Therapies, Bayer Healthcare Pharmaceuticals, Berlin, Germany

Objective: To develop a predictive mouse model for uterine fibroids.
Design: Human fibroid cells xenografted to immunodeficient mice.
Setting: University and industrial research center.
Animal(s): Immunodeficient *scid/beige* mice.
Intervention(s): Subcutaneous and intrauterine injection of fibroid-derived cells, SV40 transformation of primary cells by lentiviral transduction, proliferation determined by immunohistochemistry, FISH.
Main Outcome Measure(s): Characterization of primary and immortalized cells by Western blot and soft agar assay, determination of in vivo tumorigenicity, comparative histology and immunohistochemistry, fluorescence in situ hybridization.
Result(s): Tumorigenicity of primary myoma cells disappears upon in vitro culture. Transformation and immortalization does not restore or conserve the in vivo growth potential of cultured cells. Injection of primary cells into myometrium of mice leads to xenografts with a leiomyoma-like histology.
Conclusion(s): Primary myoma cells are suited to generate fibroid-like xenografts for studying pathogenesis without genetic modifications. In contrast, in vitro culture abolishes transplantability, and neither transformation nor immortalization is sufficient to restore tumorigenic capacity. (Fertil Steril® 2013;99:927–35. ©2013 by American Society for Reproductive Medicine.)
Key Words: Fibroid, benign tumor, xenografts, primary culture, FISH

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/droschm-uterine-leiomyomas-xenografts-fibroid/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Uterine leiomyomas (ULs) are the most common female genital tumors. According to estimations based on ultrasound examination, 70%–80% of women in their reproductive age are affected by fibroids (1). Despite their benign nature, they often cause infertility, repeated miscarriage, and extensive bleeding (2, 3). The pathogenesis is not well understood. Besides therapy with GnRH analogues or antagonists which cause only a reversible shrinkage of the tumors and elicit severe side effects, there is no effective noninvasive treatment available (4). The clonal origin of each tumor is, e.g., evidenced by typical mutations likely associated with their pathogenesis (5, 6). These include various types of recurrent chromosomal abnormalities such as deletions of the long arm of chromosome 7 and rearrangements of chromosome region 12q14∼15 (7, 8). The latter group of fibroids is characterized by a high expression of the gene encoding high-mobility-group protein AT-hook 2 (HMGA2) (9, 10). Recently, a group with mutations of the mediator complex subunit 12 gene has been identified. This seems to affect ∼70% of fibroids and appears to lead to a route of tumorigenesis different from that with *HMGA2* rearrangement (11, 12).

However, research of uterine fibroids is challenged by the need for in vivo models. At present, the most widely used model is the Eker rat or Eker rat–derived ELT3 cells (13, 14). Owing to a germ-line mutation of the tuberous sclerosis gene 2 (*Tsc2*), Eker rats spontaneously develop various neoplasms, including leiomyosarcoma and renal cell carcinoma, as well as UL-like tumors with a penetrance of 65% (13–16). Nonetheless, the *Tsc2*-driven tumor development might not reflect the sporadic pathogenesis of fibroids. Moreover, time-intensive

Received April 10, 2012; revised November 15, 2012; accepted November 16, 2012; published online December 20, 2012.
M.D. has nothing to disclose. J.B. reports occasional payment for lectures and meeting expenses by Roche Diagnostics and holds patents and receives royalties for miRNA as a biomarker. T.M.Z. owns stock in Bayer. F.P. has nothing to disclose. M.K. has nothing to disclose. N.S. has nothing to disclose.
The last two authors contributed equally to this work.
Reprint requests: Nicole Schmidt, Ph.D., Global Drug Discovery–TRG Oncology/Gynecological Therapies, Bayer Healthcare Pharmaceuticals, Muellerstraße 178, 13342 Berlin (E-mail: nicole.schmidt1@bayer.com).

Fertility and Sterility® Vol. 99, No. 3, March 1, 2013 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2012.11.032

breeding and late onset impedes the use of Eker rats as a "model on demand." The injection of Eker rat–derived ELT3 cells circumvents problems such as time-intensive breeding, but otherwise shows similar limitations (17).

Another model for UL was described by Hassan et al., who provoked growth of myoma-like xenografts by the transplantation of human tumor tissue pieces overexpressing *COX2 (*cyclooxygenase 2) and *VEGF* (vascular endothelial growth factor) through previous adenoviral transduction. Because these factors are not significantly overexpressed in leiomyomas (18, 19), this approach only partly simulates sporadic tumorigenesis.

Herein, we report an approach generating a myoma xenograft model by injection of human myoma–derived primary cells without the need for genetic manipulation and with striking similarity to human leiomyomas. This model can be used as a powerful tool to study mechanisms involved in the pathogenesis of fibroids and will open new opportunities for their treatment.

MATERIALS AND METHODS

Tissue Samples

Samples of ULs were received from various clinics in Berlin, Germany. The study was approved by the local Ethics Committee, and before surgery, informed written consent was obtained from each patient. A total number of 12 myoma tissue specimens (8 subserous, 2 intramural, and 2 submucosal) were obtained from patients with uterus myomatosus undergoing myomectomy or hysterectomy. All patients were premenopausal, and donors with hormonal or immunomodulatory therapies as well as known gynecologic comorbidities, such as endometriosis, were excluded. The surgery tissue was transferred into chilled Vispan organ conservation buffer (Bristol-Myers Squibb) and immediately shipped.

Primary Cell Isolation

Isolation of primary myoma cells was performed within 24 hours after shipment. Tissue was minced and digested with 0.25% (200 U/mL) Collagenase NB8 (Serva) in Hank buffered saline solution containing 2% fetal bovine serum (FBS) (both from Biochrome) and DNase I (Sigma) at a final concentration of 0.015%. After incubation for 2–3 hours, the cell suspension was cleaned of debris and cell aggregates with the use of a 70-μm mesh strainer (BD Bioscience), spun, and resuspended in M199 medium (PAN Biotech) containing 20% FBS and antibiotic-antimycotic solution. Cells were immediately used for in vivo experiments or seeded into 25–75-cm^2 tissue culture flasks for expansion at 37°C and 5% CO_2. When reaching confluence, cells were detached with the use of Triple-LE and subcultured in M199 medium containing 10% FBS. All media and supplements not indicated otherwise were from Invitrogen.

Cell Culture

A459 lung adenocarcinoma cells used as control cells in soft agar assay were purchased from American Type Tissue Culture and cultured in Dulbecco Modified Eagle Medium (DMEM) plus 10% FBS. The immortalized myoma cell line LM220 was generated by transfection of a subgenomic fragment of Simian virus 40 as described previously (20). The carcinoma cell lines MCF7 (DSMZ,) and HeLa-Matu cells (a kind gift from Dr. Jens Hoffmann [EPO]) were used as negative control samples in Western blotting and cultured in DMEM supplemented with nonessential amino acids, 0.2 U/mL bovine insulin, 0.1 nmol/L estradiol (Sigma), and 10% FBS. All media and supplements, unless indicated otherwise, were purchased from Invitrogen, and cells were incubated at 37°C and 5% CO_2.

Lentivirus Production and Transformation of Cells

Recombinant lentivirus was produced according to standard protocols. Briefly, the SV40 early region (SV40ER) sequence (NC_001669, bases 5,163–2,691) encoding for the oncoproteins (large T antigen and small T antigen) was cloned into pDonor221 and subsequently Lenti6/V5-Dest vectors with the use of gateway cloning according to the manufacturer's directions (Invitrogen). Expression was driven by a CMV promotor. Lentivirus was produced by cotransfection of Lenti6/V5-Dest-SV40ER plasmid and Virapower packaging mix into HEK293FT cells according to the manufacturer's directions (Invitrogen). Viral particles were harvested at 48 and 72 hours after transfection and concentrated by ultracentrifugation (2 h, 4°C, 50,000 *g*). Viral titer was determined by p24 ELISA (Perkin-Elmer). Primary cells at passage 0 were transduced at 80% confluency for 4 hours at virus concentrations of 10 μg/mL p24 in the presence of 5 μg/mL Polybrene (Sigma). Three days after infection, transduced cells were selected for 14 days with 2 μg/mL Blasticidin (Sigma).

Assay for Anchorage-Independent Growth/Soft Agar Assay

Soft agar assay was performed as described previously (21). In brief, cavities of a 6-well plate were covered with a bottom layer consisting of appropriate cell culture media mixed with ultrapure low-melting-point agarose to a final concentration of 0.6%.

After solidification, a top layer was prepared by combining cell suspension (in complete growth medium) with agarose stock solutions leading to a final concentration of 0.3% agarose and 10,000 cells per well. Mixture was immediately pipetted onto bottom layer and cultured for 14 days in a humidified CO_2 (5%) incubator at 37°C. After 14 days, wells were evaluated microscopically for growth of colonies. Assays were conducted in triplicate with cells from three independent donors.

Establishment of Xenografts by Subcutaneous Injection

All in vivo experiments were performed in accordance with German animal protection guidelines. Ovarectomized female 6–9-week-old CB-17 scid/beige mice (Taconic) were used for inoculation. Before cell injection, animals were narcotized with xylazine/ketamine (Bayer Animal Care). When reaching surgical anaesthesia, animals were treated with estrogen and