Nonobese diabetic/severe combined immunodeficient murine xenograft model for human uterine leiomyoma

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Objective: To establish a novel xenograft model using a severely immunocompromised host that is more convenient for uterine leiomyoma research compared with a pre-existing model using nonobese diabetic/severe combined immunodeficient (NOD/SCID) IL-2Rγ-null mice.
Design: Experimental study.
Setting: University and an attached animal facility.
Animal(s): NOD/SCID, SCID, BALB/c nude, and NOD/SCID IL-2Rγ-null mice.
Intervention(s): Xenografts consisting of primary cultured leiomyoma and myometrial cells in the subrenal and subcutaneous (SC) spaces in ovariectomized mice, followed by sex steroids (estrogen and P) administration.
Main Outcome Measure(s): Viability, volume, histology, and sex steroid receptor expression of xenografts in response to sex steroid administration, to evaluate feasibility of the model; and messenger RNA expression levels of 12 genes representative of leiomyoma in the xenografts, to characterize the model.
Result(s): Leiomyoma xenografts increased in volume at the highest frequency (55.1%) in response to sex steroids in NOD/SCID mice. Xenografts reproduced the histology and maintained expression of sex steroids receptors and representative genes of the original tissues. Subrenal xenografts were significantly larger than the SC xenografts, whereas those consisting of myometrial cells never increased.
Conclusion(s): The modified NOD/SCID murine subrenal leiomyoma xenograft model reproduced most characteristics of the original leiomyoma tissue. Our model provides a more convenient research tool to investigate the pathogenesis of uterine leiomyoma. (Fertil Steril® 2014;101:1485–92. ©2014 by American Society for Reproductive Medicine.)
Key Words: Leiomyoma, xenograft, NOD/SCID, animal model

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A uterine leiomyoma (UL) (or uterine fibroid) is the most common gynecologic tumor in reproductive-age women. Uterine leiomyoma–associated symptoms include menorrhagia, prolonged menstruation, and abnormal uterine bleeding (1). In addition, UL may contribute to subfertility, recurrent pregnancy loss, and complications during late pregnancy (2). Therefore, alleviation of UL-associated symptoms could provide great benefits to women's health.

Although symptomatic UL is a benign condition, surgeries such as myomectomy and hysterectomy remain the gold standard treatment, partly because there are no effective medications suitable for long-term use. Uterine leiomyoma growth is dependent on the sex steroid hormones estrogen (E) and progesterone (P) Gonadotropin-releasing hormone agonists, which completely inhibit ovarian secretion of sex steroids, are the most effective agents to reduce UL size and improve UL-associated symptoms; however, because of severe bone mineral loss, long-term use of these agents should be avoided. Recently, ulipristal acetate, a selective P receptor modulator, was marketed for the treatment of symptomatic UL in Europe (3–5). Although ulipristal

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acetate is only approved for preoperative and short-term treatment, the efficacy for symptomatic UL is similar to that of GnRH agonists, and its use does not affect circulating E levels; therefore, severe side effects have not yet been reported. Thus, ulipristal acetate may be a suitable replacement for GnRH agonists as a first-line treatment for symptomatic UL in the future.

Although the pathophysiology of UL remains largely unknown, recent genome-wide research has provided an important clue to elucidate the tumor initiation mechanism of UL. Uterine leiomyoma is considered as a monoclonal tumor, and whole-exome sequencing studies revealed that approximately 70% of ULs were due to somatic mutations in the mediator complex subunit 12 (*MED12*) gene, which codes for subunit 12 of the mediator complex (6, 7). More recently, whole-genome sequencing of UL revealed that complex chromosomal rearrangements, which may be formed by a single event of multiple chromosomal breaks and random reassembly, are common UL features (8).

Another intriguing finding associated with UL growth is the interaction between UL and the surrounding myometrium. The myometrial tissue surrounding UL, namely the pseudocapsule, has a distinct gene expression profile and angiogenic properties that may provide abundant blood supply to the leiomyoma nodule, suggesting that the interaction between leiomyoma and myometrium may transform the myometrium itself and play a pivotal role in UL growth (9).

To further investigate tumor initiation mechanisms and confirm the effect of genetic alterations in UL pathophysiology, appropriate animal models are indispensable. There are several animal UL models, which are generally divided into the following two groups: one in which the leiomyoma or leiomyoma-like tumor spontaneously develops in the uterus and/or other organs, and a second—a xenograft model—in which human UL tissues or cells are transplanted to immunocompromised hosts, mostly immunodeficient mice (10–15). We established a xenograft UL model in nonobese diabetic and severe combined immunodeficient (NOD/SCID)/interleukin-2 receptor γ-chain (IL-2Rγ)-null (NSG) mice (so-called super-immunodeficient mice) (16); NSG mice lack functional B and T cells, as well as complement activity, and exhibit reduced macrophage and natural killer cell function. In addition, they lack the IL-2Rγ chain, which is shared by multiple interleukin receptors (17). A high transplantation rate was achieved from xenografts transplanted beneath the kidney capsule, which increased in volume in response to sex steroid supplementation, thereby resembling human UL. Furthermore, RU486, a P antagonist, inhibited sex steroid–dependent growth of the xenografts. This model seems to be suitable for elucidating UL pathophysiology and investigating new agents for treatment of symptomatic UL; however, low fecundity and high cost of the mice, together with a time-consuming grafting procedure to the subrenal space that requires advanced skills, makes it difficult to implement this model in experiments that require many subjects.

Other than NSG mice, athymic nude, SCID, and NOD/SCID mice have been used as immunocompromised hosts for xenograft models (18–20). The level of immunodeficiency in these mice is lower than that in NSG mice, consequently fecundity is higher and the costs are lower; therefore, UL xenograft models using these mice may be more efficient and cost-effective. Similarly, the grafting procedure used for pre-existing models (grafting into the subrenal space) is time-consuming and requires a high skill level; thus, alternative grafting procedures are desirable.

Here we modified the UL xenograft model, which we previously established using NSG mice, and established a more convenient host model with an alternative immunologic background using a different grafting procedure. We then validated this model by comparing growth in response to hormonal treatment and gene expression profiles with those in the original tissues.

MATERIALS AND METHODS

Tissue Accumulation and Xenograft Preparation

Uterine leiomyoma and adjacent myometrial tissues were excised from the uterus during hysterectomy. Written, informed consent was obtained from all patients before surgery, and all experimental protocols were approved by the institutional review board of Chiba University, Graduate School of Medicine (Chiba, Japan). All procedures involving animals in this study were approved by the Institutional Animal Care and Use Committee of Chiba University. Procedures concerning tissue sampling, primary cell cultures, and preparation of cell pellets for the xenografts have been previously described (16, 21). Briefly, original tissues were minced and then type I collagenase and DNase I (Sigma Aldrich) aided digestion for 4–6 hours. In this study all leiomyoma and myometrial cells used were prepared from fresh surgical specimens and had never been frozen. The primary cells were cultured in Dulbecco's modified Eagle's medium/nutrient mixture F-12 (1:1) containing 5% fetal bovine serum and 1% GlutaMAX media supplement (Life Technologies) for 3–4 days, then trypsinized. We prepared cell pellets containing 5.0×10^5 primary cultured UL and myometrial cells (passage 1–2) mixed with type I collagen gel (BD Biosciences) for the xenografts and grafted them into the subrenal space and/or the SC space. Alternatively, we prepared large cell pellets containing more than double the number of cells (1×10^6 cells per pellet) to obtain an appropriate quantity of RNA and an appropriate UL-sized xenograft in the SC space.

Immunodeficient Mice

The NOD/SCID (NOD/ShiJic-scid Jcl), SCID (FOX CHASE SCID C.B-17/lcr-*scid*/*scid* Jcl), and BALB/c nude (BALB/cA Jcl-*nu*/*nu*) mice were purchased from CLEA Japan. We substituted the NSG mice that we used in a previous work with NOD/Shi-scid and IL-2Rγ-null (NOG) mice (age, 6–12 weeks; Central Institute for Experimental Animals, Kawasaki, Japan), which have a similar immunodeficient background. All experimental mice were ovariectomized before the grafting procedure.