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Animal Model

Rodent Model of Reproductive Tract Leiomyomata

Clinical and Pathological Features

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Mesenchymal tumors of the lower reproductive tract of women are poorly understood at the molecular level as a result in part of the lack of relevant animal models. The present study describes a novel model of gynecological smooth muscle tumors in which these neoplasms arise in Eker rats as part of a familial cancer syndrome. The tumors develop as a result of a germline mutation in the tuberous sclerosis 2 (TSC2) gene, and predisposition to tumor development is inherited in an autosomal dominant fashion. Uterine and/or cervical tumors arise spontaneously as single or multicentric neoplasms and increase in incidence with increasing age. The tumors were classified into three phenotypic variants of leiomyoma/leiomyosarcoma and into stromal cervicovaginal tumors on the basis of cytological and histological features and immunostaining patterns for smooth muscle actin and desmin. Tumors histologically identical to the typical human myometrial leiomyoma arose, as did a subset of atypical leiomyomas having an epithelioid phenotype. Eker rats were found to develop both benign and malignant smooth muscle tumors. The high spontaneous incidence of smooth muscle tumors of uterus and cervix in this rodent model provides a unique opportunity to study the molecular mechanisms underlying the development of these clinically important gynecological neoplasms. (Am J Pathol 1995, 146:1556–1567)

Uterine leiomyomas, usually arising from smooth muscle of the myometrium, are the most common tumor of the genital tract and occur in 20 to 25% of all women in active reproductive life.[1] Although the vast majority of uterine smooth muscle tumors in women are benign, these tumors can be associated with significant morbidity and may lead to dysmenorrhea, menorrhagia, infertility, and abortion.[2] In addition, a variety of malignant sarcomas can arise in the uterus and pose difficult problems in diagnosis and management. Although typical myometrial leiomyomas in women do not present diagnostic problems, they can have cytological and histological patterns that mimic more malignant phenotypes.[3] Despite the frequency of occurrence of genital tract smooth muscle tumors, the lack of a suitable animal model system for the study of uterine mesenchymal tumorigenesis has hampered our understanding of the pathobiology of these important neoplasms.

The diagnosis, classification, and management of uterine mesenchymal malignancy in women, particularly leiomyosarcoma, are controversial with regard to both diagnostic criteria and prognostic factors.[3] There is uncertainty about a possible link between the commonly found clinically benign uterine leiomyoma and malignant transformation of these neoplasms into

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uncommonly found sarcomatous variants.[2,4] There are also many important clinical questions concerning the effects of hormonal modulation on the growth of these neoplasms. Cellular and molecular studies of gynecological mesenchymal neoplasms would be facilitated by a relevant animal model that could be experimentally manipulated.

Although laboratory rodents have provided many useful experimental tumor systems, there have been relatively few reports of models of soft tissue tumorigenesis in these species in comparison with the numerous well characterized models of epithelial carcinogenesis. Descriptions of the induction of smooth muscle tumors in rodents include the development of mesovarian leiomyomas in rats treated with β-adrenergic stimulants such as salbutamol and terbutaline.[5] Uterine leiomyomata have been reported to develop in guinea pigs subjected to prolonged estrogen stimulation,[6,7] but these tumors were demonstrated to differ histologically from their human counterparts (reviewed in Ref. 2). The development of mesenchymal tumors of the rat genital tract is rare, although a variety of tumors including a low incidence of leiomyosarcomas have been induced by the systemic administration of potent genotoxic carcinogens.[8] Mesenchymal tumors of the uterus, principally endometrial stromal polyps and stromal sarcomas, have been reported in aged rats in the course of chronic toxicity/carcinogenicity studies, but they are an infrequent finding in most stocks and strains.[9] The only rat stock or strain with a high spontaneous incidence of reproductive tract tumors is an inbred strain of Brown Norway rats (BN/Bi). Approximately 20% of aged BN/Bi females were found to have various epithelial and mesenchymal cervical and vaginal tumors, a low percentage of which were diagnosed as leiomyomas and leiomyosarcomas.[10]

Recently, we have reported the high incidence occurrence of lower reproductive tract smooth muscle neoplasms[11] in a rat model of hereditary renal cell carcinoma first described by Eker.[12] In this animal model, Long-Evans rats that are phenotypically heterozygous for a germline mutation in a tumor susceptibility gene, recently identified as TSC-2,[13,14] develop spontaneous renal tubular epithelial tumors with an autosomal dominant pattern of inheritance. Splenic and reproductive tract mesenchymal tumors occur with high frequency as separate but associated primary tumors in a high percentage of animals that develop renal cell tumors. Homozygous wild-type Long-Evans rats that inherit two normal alleles of this gene do not develop a high incidence of these spontaneous tumors of the kidney or reproductive tract. The inheritance of two mutant alleles of the Eker gene is embryolethal.[13,15]

The objective of the present study was to characterize the pathological features and histogenesis of genital tract mesenchymal tumors in rats bearing the Eker cancer susceptibility mutation.

Materials and Methods

Animals

Female Long-Evans rats phenotypically carriers for the Eker mutation and presumably wild-type members of the same strain were studied. All rats were selectively bred from hysterectomy re-derived Eker carrier Long-Evans stock (kind gift of Dr. A. Knudson, Fox Chase Cancer Center, Philadelphia, PA). Animals were maintained pathogen-free in a barrier facility and fed NIH-07 chow (Ziegler Bros., Gardner, PA) and water *ad libitum*. Rats were housed two per filter-capped polycarbonate cage wihin mass air displacement rooms kept at 72° ± 2°F and 50 ± 5% humidity and maintained on a 12-hour light/dark cycle.

Reproductive Tract Tissues

Complete necropsies were performed on 91 female rats presumed to carry the Eker mutation. Tissues were obtained from animals used in the Chemical Industry Institute of Toxicology breeding colony (34 rats ranging in age from 10 to 27 months) and in several experimental renal carcinogenesis studies previously reported[11] in which the reproductive tracts from female animals were studied at 12 and 14 months. Tumors were aggregated from several different studies with varying numbers of female carrier animals. As carriers of the Eker mutation are predisposed to renal cell carcinoma with virtually complete penetrance, genetic status was determined by the macroscopic and/or histological presence of tumors in one or both kidneys. No macroscopic reproductive tract neoplasms have been noted to date in noncarrier Eker rats (300 female rats), demonstrating the rarity of spontaneous reproductive neoplasms in this strain. Reproductive tract tissues, including cervix, uterine body and horns, ovaries, and macroscopic lesions, were fixed in 10% neutral buffered formalin for 12 to 48 hours and then changed to 70% ethanol. Tissues were embedded in paraffin by routine methods, sectioned at 4 μ, and stained with hematoxylin and eosin (H&E). Selected sections were stained by Mallory's trichrome method for collagen and were immunostained for vimentin, cytokeratin, smooth muscle α-actin, and desmin.

Immunohistochemistry

Vimentin, smooth muscle actin, and desmin were detected by specific monoclonal antibodies (Biogenex Laboratories, San Ramon, CA) by routine immunoperoxidase methods on paraffin-embedded serial sections. Cases (see Table 3) were selected to represent the full spectrum of histological phenotypes. Briefly, deparaffinized sections were treated with 3% hydrogen peroxide solution for 10 minutes, followed by two 5-minute washes in phosphate-buffered saline (PBS) (pH 7.6). Sections were next incubated for 10 minutes with normal goat serum (Vector Laboratories, Burlingame, CA) diluted 1:60 in PBS. The tissue sections were exposed to primary antibodies diluted in PBS containing 0.5% bovine serum albumin for 1.5 hours at room temperature (anti-vimentin, 1:1000; anti-smooth muscle actin, 1:50; and anti-desmin, 1:50). After antibody incubation, sections were washed twice in PBS and treated for 30 minutes with biotinylated anti-mouse immunoglobulin G (Vector Laboratories). After washing in PBS, sections were treated with streptavidin horseradish peroxidase (Zymed Laboratories, San Francisco, CA) for 30 minutes. Slides were washed in PBS, rinsed in distilled water, and treated with 3-amino-9-ethylcarbazole for 5 to 7 minutes (Zymed Laboratories), and then counterstained with hematoxylin (Biomedica Corp., Foster City, CA).

Tumorigenicity in Nude Mice

Eker rat tumor-derived cell line ELT 3[16] was inoculated into four 8- to 10-week-old intact female BALB/cAnNCr-nu/nu mice (National Cancer Institute, Frederick, MD). Cells were injected subcutaneously in the right and left suprascapular regions at 1×10^6 and 5×10^6 cells/site. Before inoculation, cells were harvested in log phase growth and resuspended in 0.1 to 0.2 ml of serum-free medium. Mice were sacrificed by CO_2 inhalation when tumors reached 1.0 cm in diameter. A portion of each tumor was frozen at −70°C for subsequent analysis, whereas the remainder of the sample was fixed in 10% neutral buffered formalin for histopathological examination. Samples were embedded in paraffin by routine methods, sectioned, stained with H&E, and immunostained for desmin and smooth muscle actin.

Results

Clinical Findings

The rat leiomyomas appeared as circumscribed or pedunculated (multi)nodular masses ranging in size from 5 mm to 8 cm in diameter, generally found on the dorsal aspect of the partially fused uterine horns at the cervico-uterine junction (Figure 1A). Although the majority of the tumor masses were present in the region of the cervix and fused uterine horns (see Figure 6A), occasional tumors were noted on one or both uterine horns. Uterine masses presented as mural thickenings and occasionally as pedunculated masses or polypoid projections distorting the myometrial wall (Figure 1B). The usual appearance was of a solitary, firm, gray mass although occasionally multifocal cervical and/or uterine masses (11 of 47 14-month-old animals) were present. Rarely (3 of 47 14-month-old animals), vaginal masses were present as smooth, firm, gray, polypoid projections into the vestibule.

Few clinical signs were attributed to the uterine or cervical masses, although occasionally a hemorrhagic pedunculated mass protruded from the vagi-

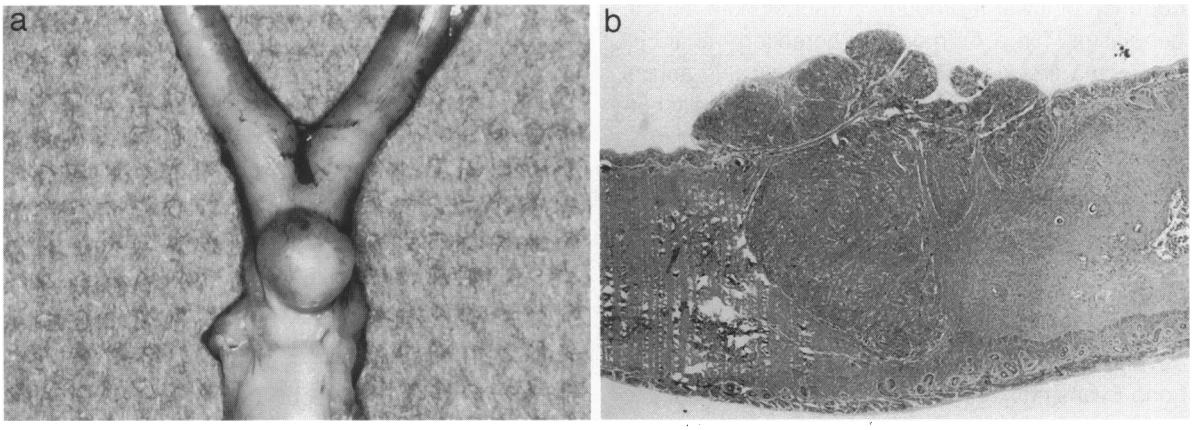


Figure 1. A: *Nodular epitheliod leiomyoma arising at the usual site of origin at the cervico-uterine junction.* **B:** *Fascicles of fusiform cells comprise a typical variant arising from the uterine myometrium and forming a multinodular polyploid transmural mass. H&E, ×15.*

nal canal, necessitating euthanasia of the animal. A few moribund animals were noted to have large abdominally palpable masses that had evidence of infarction and necrosis. Foci of hemorrhage and necrosis were present in masses larger than 3 cm in diameter. Fibrous adhesions to the colon and urinary bladder were sometimes found in association with these large masses and resulted in clinical evidence of urinary incontinence and constipation.

Macroscopic tumors of the uterus and cervix have been detected in female Eker rats as early as 10 months of age. Tumor incidence increases with increasing age, although colony animals generally are not kept beyond 18 months of age, at which time they tend to succumb to uremic complications of renal tumors and chronic progressive nephropathy. Examination of reproductive tracts from a group of 12-month-old female rats phenotypically carrying the Eker mutation revealed that 36% had macroscopic uterine and/or cervical masses (Table 1). Microscopically, 8 of 13 (62%) of a group of 12-month-old Eker carrier animals (as determined by the presence of kidney neoplasms) had uterine and/or cervical lesions. In 14-month-old animals, the incidence of grossly visible uterine lesions rose to 43%, whereas 72% of animals had lesions visible by light microscopic examination (Table 1). The finding of smooth muscle tumors is primarily limited to the lower reproductive tract, as only a single smooth muscle tumor has been found (intestinal leiomyosarcoma) at another anatomic site in 91 female carrier animals examined.

Histological Findings

Three distinct histopathological patterns were discernible in the reproductive tract leiomyomas examined and include typical, epithelioid, and mixed appearances (Table 2). Tumors depicted to have a typical leiomyoma appearance were comprised of relatively well differentiated smooth muscle cells arranged in interwoven bundles and fascicles (Figure 2, A and B). The elongated, fusiform cells with eosinophilic cytoplasm contained slender vesicular nuclei, generally with inconspicuous nucleoli. The typical fusiform leiomyomas occasionally showed foci of hypercellularity characterized by cytoplasmic basophilia and/or nuclear and cellular pleomorphism. A relatively low mitotic index (0 to 2/10 HPF) was generally noted in this group of tumors. The histological pattern termed typical was most often noted associated with masses present on the uterine horns and appeared to arise from either the outer longitudinal or inner circumferential smooth muscle layers of the myometrium (Figure 1B). Trichrome staining revealed relatively small amounts of collagenous connective tissue stroma (see Figure 6C).

Table 1. *Incidence of Uterine and Cervical Smooth Muscle Tumors*

	12 Months	14 Months
Macroscopic	5/14 (36%)	19/43 (43%)
Microscopic	8/13 (62%)	31/43 (72%)

Table 2. *Tumor Diagnosis by Site*

	Location		
Classification	Uterus	Cervix	Vagina
Leiomyoma			
Typical	9		
Epithelioid	5	16	3
Mixed	14	6	
Leiomyosarcoma			
Typical	2		
Epithelioid		1	
Mixed	1		
Stromal Tumor		1	3

In contrast to the fusiform typical leiomyomas, the epithelioid variants were composed of round to stellate cells with indistinct cell margins and relatively sparse, pale, eosinophilic, sometimes vacuolated cytoplasm (Figure 3). Round to vesicular nuclei often contained a single prominant nucleolus (Figure 3B). As with the typical fusiform leiomyomas, the epithelioid tumors were generally infiltrative into surrounding tissues and did not have evidence of encapsulation or circumferential hyalinization. Mitotic activity in the epithelioid leiomyoma variant was generally low (<1/10 HPF). These tumors rarely demonstrated nuclear atypia. Occasionally a cluster of cells of the epithelioid variant appeared within a mass comprised of typical leiomyomatous cells (see Figure 6B). The epithelioid tumors were generally well vascularized even when extremely small in size. Often, the tumor cells were aggregated in a perithelial pattern surrounding capillaries. Thick-walled arterioles were occasionally noted within tumor boundaries. Areas of collagen deposition, including hyalinization, were often present separating polygonal cells in the epithelioid tumors (Figure 3B). The epithelioid variants ranged from solid masses of highly cellular sheets of epithelioid cells to paucicellular regions in which individual stellate cells were separated by abundant hyalinized collagen.

The epithelioid tumors predominated in the cervical and vaginal sites (Table 2). Large epithelioid tumors were found within the uterine wall but in all cases were found to infiltrate an enlarged uterocervical region that was presumed to be the site of origin. Mi-

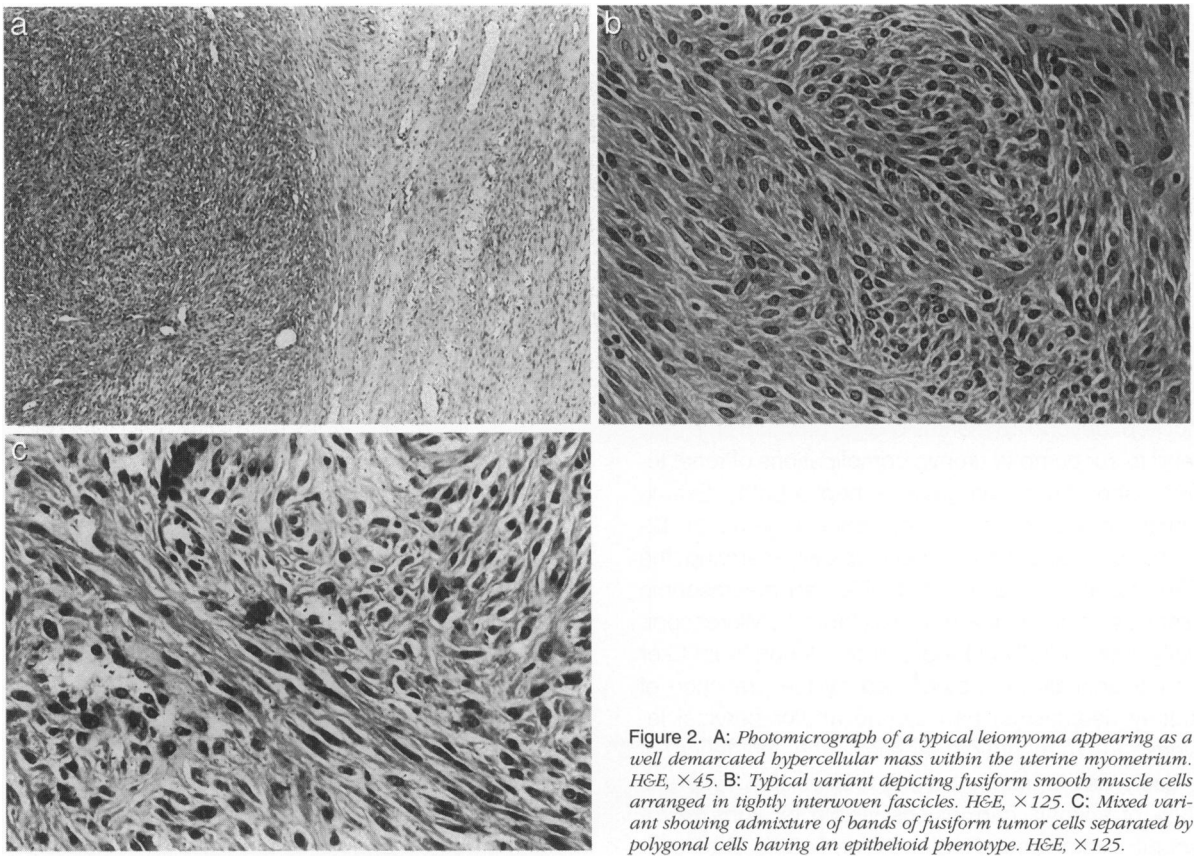


Figure 2. A: *Photomicrograph of a typical leiomyoma appearing as a well demarcated hypercellular mass within the uterine myometrium. H&E, ×45.* B: *Typical variant depicting fusiform smooth muscle cells arranged in tightly interwoven fascicles. H&E, ×125.* C: *Mixed variant showing admixture of bands of fusiform tumor cells separated by polygonal cells having an epithelioid phenotype. H&E, ×125.*

croscopic examination of uterocervical junctions revealed that these leiomyoma variants tended to arise in a characteristic region where the two partially fused uterine horns joined the cervix. Usually it was impossible to discern whether these tumors arose within the cervical stroma or from vestiges of the inner layer of myometrium. The earliest microscopic foci of epithelioid leiomyoma were generally found at the interface of the myometrium and the stroma at the uterocervical junction. These earliest microscopic tumors had cytological features, extracellular matrix characteristics, and vascular patterns identical with those of the larger masses that distended the uterine horns. Vaginal epithelium overlying subjacent cervical and/or vaginal epithelioid leiomyomas was noted in some cases to be severely hyperplastic (see Figure 5B).

A commonly found histological pattern consisted of a mixed variant that shared features of the typical fusiform leiomyomas and the epithelioid tumors (Figure 2C). A few of these tumors exhibited nuclear pleomorphism in the fusiform cells, although the mitotic index was generally low. The mixed variant was generally found in the same location as the epithelioid tumor at the uterocervical junction. These tumors often consisted principally of round to polygonal epithelioid cells and had the identical prominent vascular pattern of the epithelioid variant.

Four uterine smooth muscle neoplasms were classified as leiomyosarcomas primarily on the basis of their extreme nuclear atypia and cellular pleomorphism (Figure 4). Two of these tumors were typical fusiform leiomyosarcomas, one was a mixed variant, and one was an epithelioid tumor. The typical variants were composed of anaplastic fusiform cells with marked nuclear atypia and pleomorphism and a relatively low mitotic index (<5/10 HPF). The hypercellular areas consisted of basophilic fusiform smooth muscle cells arranged in broad fascicles. One of these tumors metastasized to the lung via hematogenous spread and formed micrometastatic nodules of relatively well differentiated fusiform smooth muscle cells (Figure 5A).

Four stromal polyps were found in the cervical region of animals that had one or more leiomyomas diagnosed. These tumors appeared to arise from the stroma of the epithelium-covered fibromuscular cushions (*portio vaginalis uteri*) that project into the vaginal lumina from the external openings in the cervix.

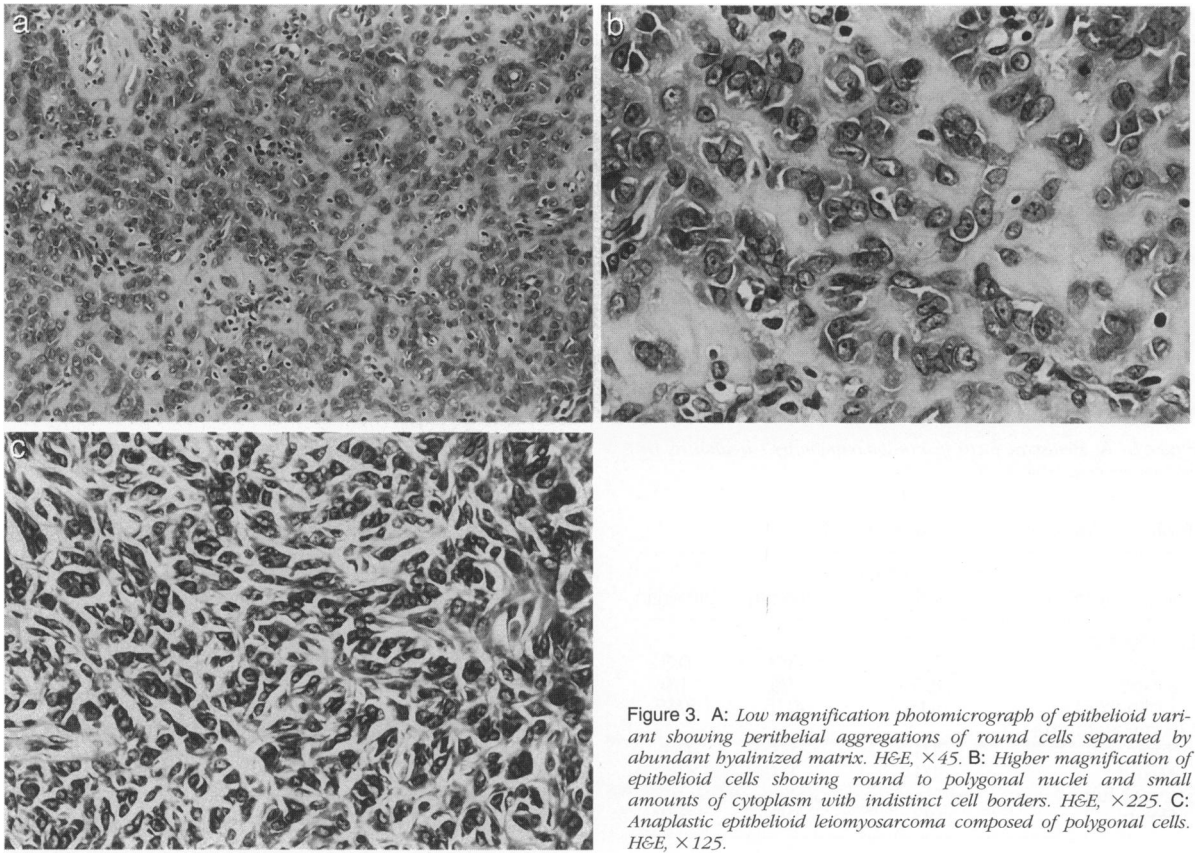


Figure 3. A: *Low magnification photomicrograph of epithelioid variant showing peritbelial aggregations of round cells separated by abundant hyalinized matrix. H&E, ×45.* B: *Higher magnification of epithelioid cells showing round to polygonal nuclei and small amounts of cytoplasm with indistinct cell borders. H&E, ×225.* C: *Anaplastic epithelioid leiomyosarcoma composed of polygonal cells. H&E, ×125.*

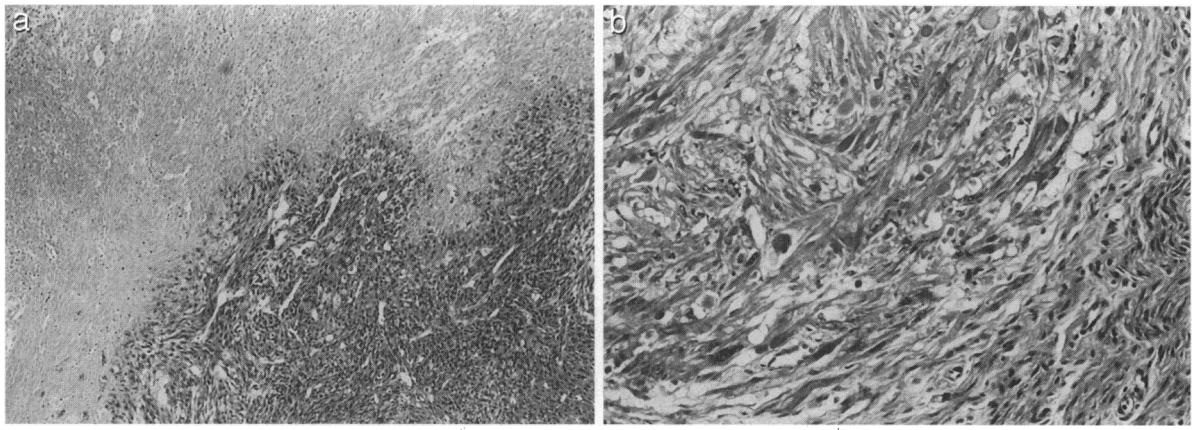


Figure 4. A: *Photomicrograph of leiomyosarcoma having a typical fusiform phenotype with a sharply demarcated area of infarction. H&E, ×45.* B: *Loosely arranged fascicles of anaplastic cells within a typical leiomyosarcoma. H&E, ×125.*

Immunohistochemical Studies

Table 3 summarizes the results of immunostaining for smooth muscle actin, desmin, and vimentin. The typical leiomyomas and leiomyosarcomas arising in the uterine myometrium were strongly immunoreactive for smooth muscle actin and desmin that usually stained 25 to 50% of tumor cells (Figure 6). The mixed tumors were generally immunoreactive for smooth muscle actin and desmin in the fusiform but not epithelioid cells. Immunoreactivity intensity and distribution were generally diminished in these tumors in comparison with the group of typical leiomyomas. Unlike the fusiform cells of the typical variants, the epithelioid tumors did not generally immunostain for the presence of smooth muscle actin or desmin. Several of these

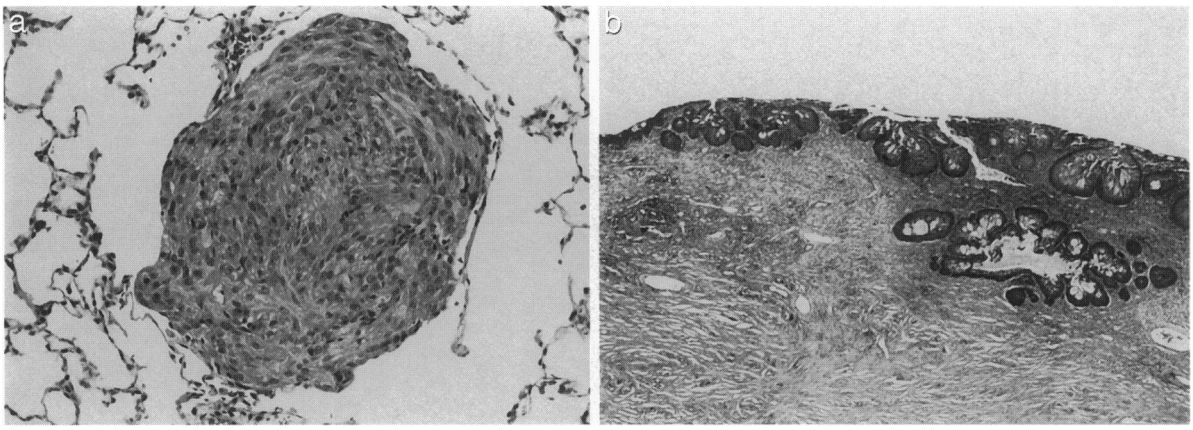


Figure 5. A: *Metastatic focus within the pulmonary vasculature. H&E, ×125.* B: *Hyperplastic squamous epithelium overlying a submucosal vaginal leiomyoma. H&E, ×45.*

Table 3. *Immunohistochemistry Results*

Tumor diagnosis	Smooth muscle actin	Desmin	Vimentin
Leiomyoma			
Typical	3/3	3/3	0/3
Mixed	8/9	7/9	1/9
Epithelioid	5/15	4/15	8/15
Leiomyosarcoma			
Typical	1/1	1/1	0/1
Mixed	1/1	1/1	0/1
Stromal Tumor			
Polyp	0/3	0/3	3/3

tumors did, however, demonstrate strong cytoplasmic immunoreactivity for smooth muscle actin and desmin (Figure 6, D and G). In general, smooth muscle actin immunoreactivity was present in a greater number of tumor cells than was desmin immunoreactivity for all phenotypes examined. Vimentin immunostaining was not present in any of the differentiated smooth muscle cells in either typical or mixed leiomyomas. Of the epithelioid tumors, approximately one-half had immunoreactivity to vimentin (Figure 6H). The group of stromal polyps examined lacked immunoreactivity to smooth muscle actin or desmin but did stain for vimentin.

The two leiomyosarcomas had staining patterns very similar to those of leiomyomas. The fusiform sarcoma immunostained strongly for desmin and smooth muscle actin (Figure 6F), whereas the mixed sarcoma was positive in the fusiform cells and negative for expression of these proteins in the epithelioid cells of this tumor. Metastatic foci in the lungs showed immunoreactivity to both smooth muscle actin and desmin but not to vimentin, the same pattern noted in one of the primary sarcomas present in the animal in which the metastasis was observed.

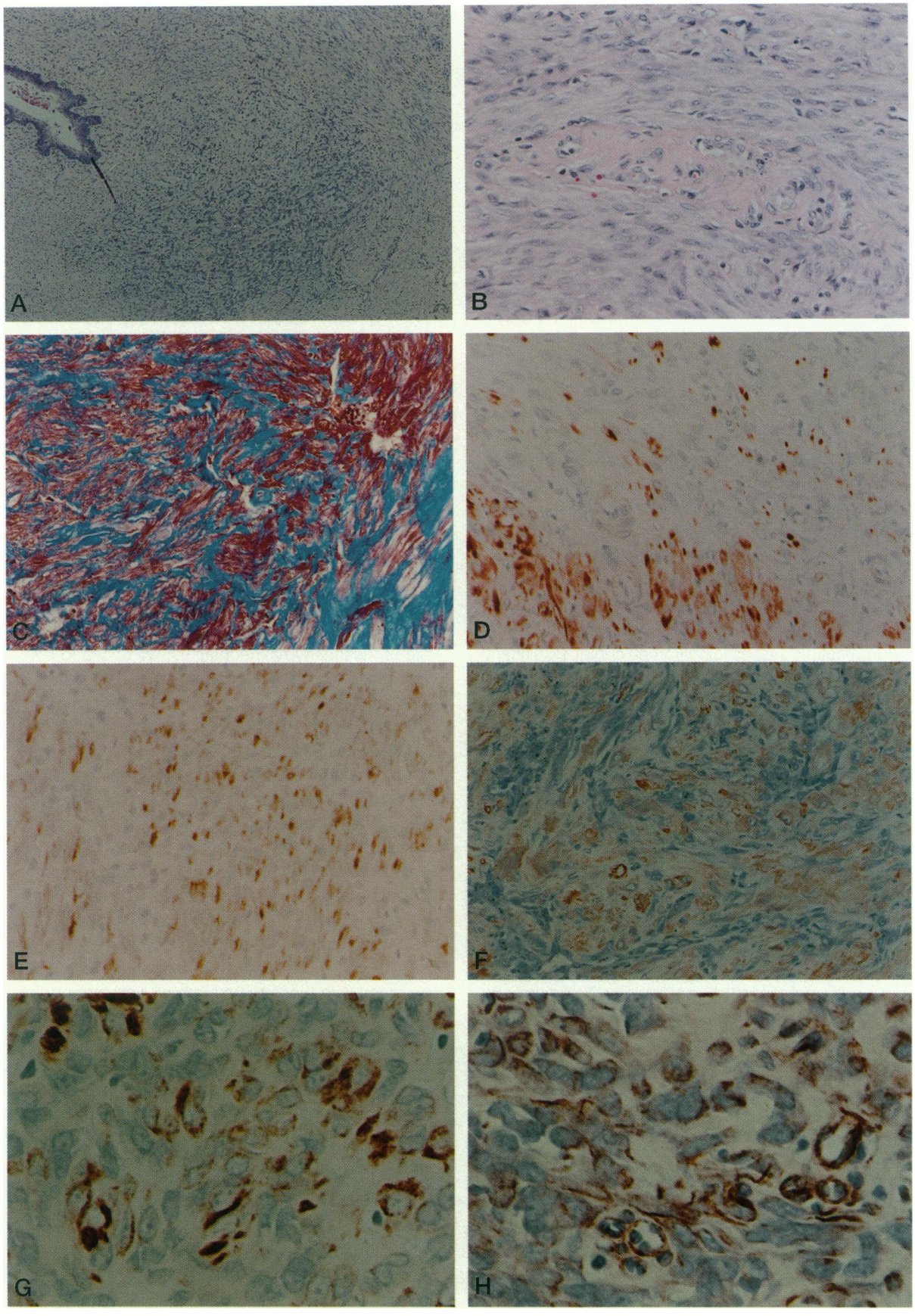
Tumor Xenografts

Retention of the ability of the epithelioid tumor variants to express a more typical smooth muscle phenotype was confirmed with a cell line (ELT 3) derived from an epithelioid leiomyoma.[16] ELT 3 is tumorigenic in nude mice, and histological examination of the nude mouse tumors produced by these cells was used to determine whether the epithelioid variants in Eker rats retained the ability to differentiate into tumors exhibiting a more typical histology. ELT 3 tumors (Figure 7) formed masses composed of highly anaplastic tumor cells exhibiting extreme nuclear and cytoplasmic pleomorphism. In regions, the neoplastic cells appeared fusiform with ovoid nuclei and were arranged in a fascicular pattern with a sarcomatous appearance. Karyomegaly and multinucleate giant cells were prevalent. Numerous mitotic figures including abnormal tripolar forms were evident. The tumor also stained positively for smooth muscle α-actin expression by immunohistochemical methods (data not shown).

Discussion

In the present studies, it was found that a high proportion of Long-Evans rats diagnosed as putative carriers of the Eker mutation developed uterine and/or

Figure 6. A: *Basophilic epithelioid leiomyoma cells at uterocervical junction forming an early microscopic focus. H&E, ×60.* B: *Island of epithelioid differentiation within fascicles of typical leiomyoma. H&E, ×300.* C: *Trichrome stain of typical uterine leiomyoma showing separation of fusiform cells by dense collagenous connective tissue (×150).* D: *Immunoreactivity to smooth muscle actin in epithelioid leiomyoma (×150).* E: *Immunoreactivity to smooth muscle actin in typical leiomyoma (×150).* F: *Smooth muscle actin immunoreactivity in a typical leiomyosarcoma. (×150).* G: *Desmin immunostaining of epithelioid variant (×300).* H: *Vimentin immunoreactivity in epithelioid variant. (×300).*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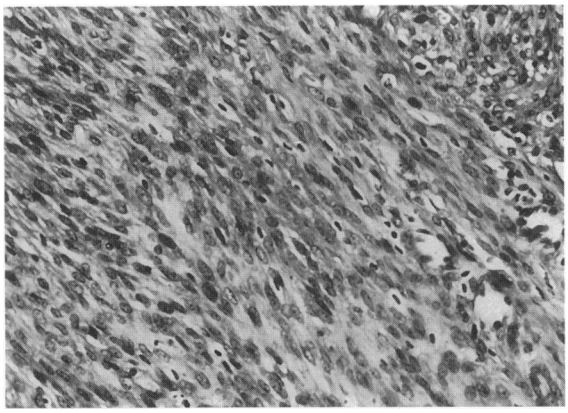

Figure 7. *Photomicrograph of tumor arising in nude mouse injected with epithelioid variant. Mass consists of anaplastic fusiform cells arranged in fascicles. H&E, ×125.*

cervical smooth muscle neoplasms at a young age. The Eker rat is the only available model system in which these tumors develop spontaneously with a high frequency and should prove invaluable in helping to elucidate the biology and responses of these clinically important tumors. Uterine leiomyomas develop in a large percentage of women, and it is estimated that between 25 and 50% of these tumors are symptomatic.[2] Just as leiomyomas confer significant morbidity and can affect fertility in the women in which they develop, to date, only one uterine tumor-bearing Eker rat has produced a viable litter (1 litter of 15 bred as compared with five litters born to a cohort of 25 non-tumor-bearing littermates bred at 7 months of age).

The Eker rat gynecological smooth muscle tumors can be classified into three distinct histological appearances, consisting of typical, epithelioid, and mixed variants of leiomyoma. These tumors demonstrate immunohistochemical staining patterns similar to those found in smooth muscle tumors of women.[17,18] The histological classification of the tumors in the present study, particularly the epithelioid and mixed variants, poses difficulty because there are few comprehensive studies of mesenchymal reproductive tract neoplasms or smooth muscle tumors in rats as a result of their low spontaneous and induced incidences. The present study is one of the largest collections of uterine and cervical mesenchymal neoplasms reported in rats. Although it is often difficult to directly compare rat and human tumors, the typical fusiform smooth muscle tumors have been reported to be phenotypically identical between the two species,[19] and the typical variant of leiomyoma and leiomyosarcoma diagnosed in the present study is identical to that described in humans.[20] In both rats and humans, these tumors appear to arise from the myometrial smooth muscle.

In contrast to women in which most uterine leiomyomas are of typical fusiform smooth muscle morphology, a relatively high proportion of Eker rat uterine neoplasms had an atypical epithelioid appearance. These tumors resemble a relatively rare type of atypical smooth muscle tumor in women termed the uterine epithelioid leiomyoma.[21,22] The epithelioid tumors found in the cervix and vagina of Eker rats seem identical to cervicovaginal tumors that have been previously described in BN/Bi rats by Burek and coworkers[10] and termed round cell sarcomas, although these authors later suggested that these were in fact a variant of leiomyosarcoma.[23]

The rarity of uterine leiomyomas in rats makes differentiation of benign leiomyoma *versus* malignant leiomyosarcoma a difficult exercise. In the present study, almost all tumors were diagnosed as benign leiomyomas on the basis of a lack of nuclear and cellular atypia and lack of extrauterine invasion or metastasis. With the exception of a single tumor with hematogenous metastases to the lung, none of the uterine or cervical smooth muscle tumors invaded locally into vessels or into adjacent structures. Even in the presence of local tissue invasiveness characteristic of mesenchymal neoplasms, we chose to diagnose these tumors as benign because of the lack of nuclear atypia, a lack of distal mestastasis, a lack of vascular invasion or spread into adjacent structures, and low mitotic indices.

In women, mitotic activity of uterine leiomyomas is believed to be an important indicator of malignancy.[24] No clinical information is available, however, for the rat. Interestingly, a benign appearing Eker rat leiomyoma had the highest mitotic rate of any tumor in the study, whereas, in comparison, a leiomyosarcoma with severe nuclear atypia and hematogenous metastases had a relatively low mitotic index. The pitfalls of mitotic index data as a criterion of malignancy in women with these tumors are well described and include the fact that mitotic activity may vary with the phase of the estrous cycle.[25] The Eker rat model should prove useful for studies that examine how hormonal milieu may affect the mitotic activity and the growth of uterine leiomyomas.

Recently, Hino and colleagues confirmed our previous findings of uterine mesenchymal tumors as extra-renal primary neoplasms in Eker rats.[11,26] They suggested that the tumors were uterine sarcomas of probable stromal origin but did not conduct studies to elucidate tumor histogenesis. Controversy regarding the classification of some of these tumors, particularly the epithelioid variants, is understandable. The his-

togenetic classification of uterine mesenchymal neoplasms is known to be problematic,[3] and smooth muscle differentiation can occur in a variety of endometrial stromal lesions.[27] There are several features of the tumors described in the present study as epithelioid variants that resemble endometrial stromal tumors described in women, such as abundant hyaline collagen in a paucicellular environment of polygonal cells having inconspicuous cytoplasm and arrangement of these cells around a prominent network of arborizing, occasionally thick-walled vessels. Despite the atypical smooth muscle appearance of the epithelioid variant and the finding of certain histological features shared with endometrial stromal tumors of women, the present studies and those of Howe et al[16] characterizing cell lines derived from these neoplasms strongly support a diagnosis that the epithelioid variants are related to the typical leiomyomas and are of smooth muscle origin.

We base this conclusion on several lines of evidence, including (1) immunohistochemical findings that a small population of the epithelioid tumors strongly immunostain for the presence of smooth muscle actin and desmin, (2) mixed variants in which there is apparent transition of epithelioid cells into classical fusiform smooth muscle cells, (3) differentiation into fusiform smooth muscle cells and immunoreactivity for smooth muscle α-actin in a tumor arising in an immunodeficient nu/nu mouse injected with cell line ELT 3 that was established from an epithelioid leiomyoma variant, (4) clear expression of smooth muscle actin and desmin by this same cell line,[16] and (5) morphology differing from the commonly found endometrial stromal tumors that have been described in rats.[28] The conclusion that the epithelioid mesenchymal tumor is a smooth muscle tumor variant is supported by earlier studies of Burek, who described transplantation studies in which two cervicovaginal "round cell sarcomas" of aged BN/Bi rats (histologically identical with the epithelioid mesenchymal tumors of the present study) were transplanted into 6-week-old female syngeneic recipients. The transplanted tumors grew as elongated spindle cells and resembled leiomyosarcomas rather than the round-cell pattern of the primary tumors.[23]

The site specificity for lesion development of the epithelioid variant at the uterocervical junction is interesting. It may be that smooth muscle tumors of the rat cervical and/or vaginal region are influenced by stromal or hormonal factors to assume an epithelioid phenotype. Many of the larger epithelioid neoplasms that affected the uterine myometrium had a more fusiform appearance, with streaming of epithelioid cells into the mixed or transitional phenotype. The characteristic region of tumor origin at the cervico-uterine junction makes this an attractive animal model for studies of mesenchymal tumor histogenesis. In the present study, neither phenotypically distinct preneoplastic lesions nor regions of malignant transformation within benign tumors were noted. Nonetheless, it will be useful and interesting to characterize potentially important genetic alterations within small microscopic foci and compare them with changes within large uterine and cervical masses.

The benign stromal polyps found in the vagina of Eker rats in the present study are similar in histological appearance and immunostaining pattern to those previously reported in rats.[29] Because these are relatively commonly found in strains of aged rats, including Long-Evans, it is premature to assume that stromal tumors are associated with the Eker mutation. Renal cell tumors, splenic vascular tumors, and uterine and cervical smooth muscle tumors are extremely uncommon tumor types in Long-Evans or other common stocks or strains of rats and are thus clearly identifiable with the Eker familial cancer syndrome.[11] It is possible that, after analysis of large numbers of carrier and wild-type Eker rats is conducted, other tumor types commonly found in Long-Evans rats, such as endometrial and/or vaginal stromal tumors or pituitary tumors, will prove to be associated with the Eker mutation as has been suggested by others.[26]

To date, the Eker rat is the only animal model reported to develop a high spontaneous incidence of gynecological leiomyomata. A line of German shepherd dogs reported to have hereditary renal cell carcinoma was also found to have a high incidence of spontaneous multicentric uterine leiomyomas as part of a familial cancer syndrome.[30] The genetics underlying the canine syndrome are as yet completely unknown, as is any potential role for the TSC2 gene. Interestingly, these dogs develop collagenous dermal nodules having some similarity to human lesions of tuberous sclerosis. It will be of interest to examine the role of the human TSC2 gene in gynecological leiomyomata. Development of leiomyomas in individuals with germline TSC2 alterations may go unnoted because of the high spontaneous incidence of the tumor type. In addition, individuals afflicted with tuberous sclerosis tend to die at an age that is much earlier than the ages at which leiomyomata typically arise. There are no reports of TSC2 mutations in spontaneous leiomyoma development to date, but future studies will help to determine whether alterations in this gene contribute to tumor development in women.

Until recently, there has been relatively little interest in the molecular mechanisms underlying the development of human soft tissue tumors, and there have

been relatively few studies of the genetic basis of leiomyomas. A variety of inherited predispositions to soft tissue tumors are known, in most of which susceptibility to tumor development is inherited in an autosomal dominant fashion similar to that seen in the Eker rat. Presently the molecular mechanism(s) by which the underlying germline genetic abnormalities prediposes to oncogenesis are in most cases unknown.[31] The Eker rat should provide a useful tool in which to examine the molecular and cellular basis of smooth muscle tumorigenesis in a well characterized system. These rats should also provide a useful animal model in which to examine the interaction of hormonal environment on the growth and differentiation of smooth muscle tumors of the genital tract. Certain important clinical questions can be addressed with *in vivo* studies, including the effects of obesity on the development of myometrial tumors[32] and the effects of hormones and antihormones on the growth and development of reproductive tract leiomyomata. Cell lines established from Eker rat leiomyomas and leiomyosarcomas have been found to variably express estrogen and progesterone receptors and should provide a useful system in which to examine the effect of steroid hormones and hormone receptors on the development of smooth muscle tumorigenesis.[16]

In summary, the Eker rat familial cancer syndrome provides a high incidence animal model of gynecological smooth muscle tumorigenesis. The tumors that arise have many similarities to their human counterparts as well as some interesting phenotypic differences. This animal should provide a useful experimental tool in which to investigate fundamental questions of soft tissue tumor pathobiology.

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