Estrogen Receptor-Alpha (ER-α) Immunoexpression in Uterine Leiomyomas and Matched Myometria in Potbellied Pigs (*Sus Scrofa*)

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Abstract

BACKGROUND/OBJECTIVE: Uterine leiomyomas (fibroids) commonly occur in adult female potbellied pigs 5 years of age and older. The pathogenesis of fibroids in potbellied pigs and women remains unknown; however, it has been reported in the literature, that in women, these tumors are hormone dependent. Prominent ER-α staining has been reported in the myometrium of the sow during specific stages of the estrous cycle, but little data exists on the presence of this receptor in potbellied pig leiomyomas. In this study, we determined ER-α expression in porcine tumors versus matched myometria.

METHODS: Immunohistochemistry was performed on formalin-fixed tissues obtained from potbellied pigs. Semi-quantitation of ER-α was based on the staining intensity (range=0-4) and statistical analysis of scores for positively staining nuclei in uterine leiomyoma and myometrial cells.

RESULTS: ER-α was localized to the nuclei of tumor and myometrial cells and staining intensity varied among tumors. The overall intensity scores for the leiomyomas were higher than those for myometrial samples, similar to that observed in women. However, statistical analyses revealed no significant difference (p=0.50) in staining intensity for pig tumors compared to matched myometria. The lack of statistical significance is likely due to low power as a result of the small sample size. The majority of pigs with data on both tissues had higher average scores for the tumor than for normal myometrium; if this tendency continues in a larger sample, we would expect to find a significant difference. (Note: subsequent to submission additional samples were evaluated, and as expected, a significant difference was observed.)

CONCLUSIONS: The findings support further investigations into using the potbellied pig as a suitable animal model for studying fibroids in women, as well as understanding the regulation of ER-α expression in tumors during the estrous cycle.

Introduction

Uterine leiomyomas, or fibroids, clinically affect at least 25% of American women and result in almost 200,000 hysterectomies each year. Subclinical incidence is as high as 77% among American women. The tumors tend to occur during the third and fourth decade of life, are more common in nulliparous individuals, and may be single or multiple. Potbellied pigs likewise develop uterine leiomyomas during the prime reproductive years, and clinical, gross (Figure 1), and histologic features (Figure 4) are similar to those reported for fibroids in women.

Features such as a tendency to increase in size during pregnancy and regression following menopause indicate hormone responsiveness, but little is known of the pathogenesis of development and growth of uterine leiomyomas. In women, the role of steroid hormones such as estrogen and progesterone has been evaluated via blood hormone measurements, RNA levels, and gene expression as well as immunohistochemical quantification of specific steroid hormone receptors in uterine leiomyomas and matched myometrium. Conflicting data have been reported in the literature, but the majority indicate that estrogen receptor alpha (ER-α) levels are elevated in fibroids versus normal tissue. Prominent ER-α staining has been reported in the myometrium of the sow during specific stages of the estrous cycle, but little data exists on the presence of this receptor in potbellied pig leiomyomas. This retrospective study was intended to further evaluate uterine leiomyomas versus matched myometrium in the potbellied pig to determine ER-α expression.

Materials and Methods

Formalin-fixed, paraffin-embedded tissues were sectioned at 4-5 µm. Immunohistochemistry was performed by the avidin-biotin-peroxidase technique [Vectastain Elite avidin-biotin immunoperoxidase reagents (Vector Labs, Burlingame, CA)]. Tissue slides were deparaffinized in xylene and alcohols and placed in a 3% hydrogen peroxide solution for 15 minutes. Antigen retrieval was achieved by placing the tissue sections in 1X citrate buffer, pH 6.0 (Biocare Medical, Walnut Creek, CA) and boiling the tissue slides at a high temperature in the decloaker. After cooling for 10 minutes, mouse anti-ER-α antibody (1:300 dilution, Coulter/Immunotech, Miami, FL) was applied for 1 hour in a humidifier chamber. Secondary antibody and label were similarly applied for 30 minutes each as directed [Vectastain elite kit (Vector Labs, Burlingame, CA)]. Liquid DAB (3,3-Diaminobenzidine Tetrahydrochloride, Carpinteria, CA) was used as a chromogen, and the slides were counterstained with a modified Harris hematoxylin (Richard Allen, Kalamazoo, MI). Slides were dehydrated and coverslipped with Permount (Surgipath, Richmond, IL).

Tissues were evaluated for staining intensity of the nuclei and scored using a scale of 0-4 (0 = negative, 1 = minimal, 2 = mild, 3 = moderate, 4 = intense). Each tumor and adjacent normal myometrium was separately scored (see Figure 2, inset).

Statistical Analysis: Scores were analyzed using both the tumor and the animal as the unit of analysis. In both cases, the differences between tumor scores and matched myometrium scores were not normally distributed, and two-sided paired Wilcoxon signed tests were performed on the difference scores. Using the tumor as the unit of analysis, there was a significant difference between the tumor score and the matched myometrium score ($p = 0.0034$). Using the animal as the unit of analysis, there was also a significant difference ($p = 0.0256$). In both analyses, the tumor score tended to be higher than the matched myometrium score.

Note: In the original abstract, there was no significant difference reported due to low power as a result of the small sample size. Subsequent to submission additional samples were evaluated, and as expected, a significant difference was observed.

Results

A total of 17 uterine leiomyomas in five potbellied pigs were evaluated. Staining intensity varied widely among individuals as well as between tumors in the same animal. Staining ranged from minimal to intense, and normal myometrium stained more consistently than tumor tissue within a given animal (Figures 3 - 6). Despite variation, the leiomyomas yielded an overall increase in staining intensity as compared with adjacent myometrium (Figure 2).

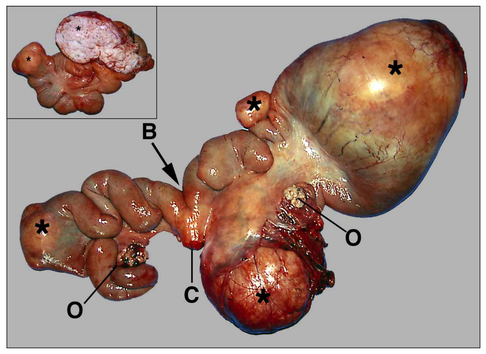

Figure 1. Porcine uterus with four leiomyomas (*) within the uterine horns; the cervix (C), uterine bifurcation (B), and both ovaries (O) are identified. **Inset:** Cut surface of one porcine leiomyoma.

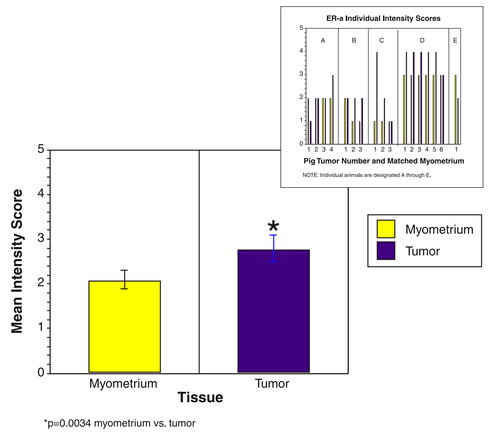

Figure 2. Estrogen Receptor Alpha (ER-α) Mean Intensity Scores for Uterine Leiomyomas and Matched Normal Myometrium from Potbellied Pigs *(Sus Scrofa)*

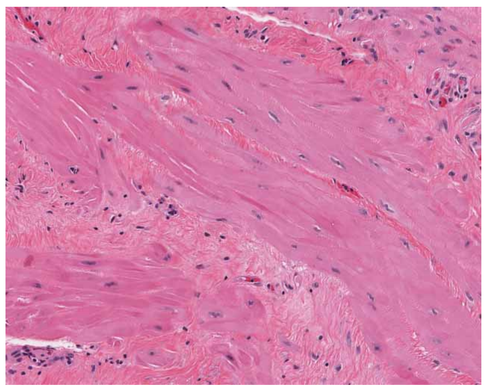

Figure 3. H & E stained section of normal porcine myometrium. 20X, original magnification.

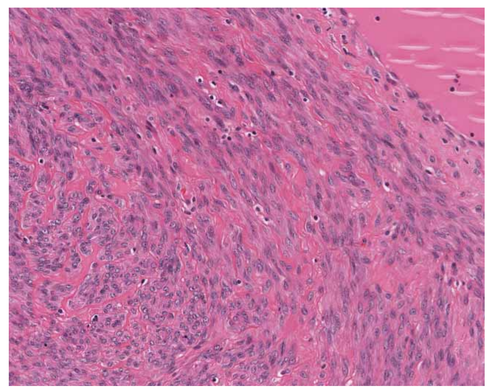

Figure 4. H & E stained section of a porcine uterine leiomyoma. 20X, original magnification.

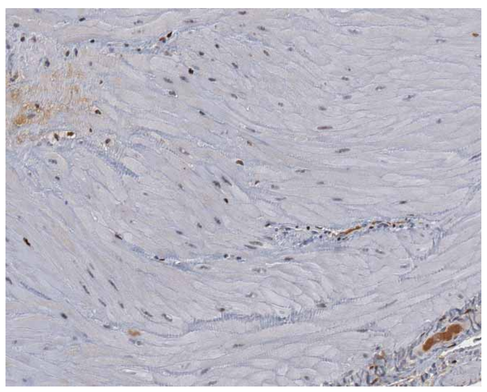

Figure 5. Mild immunolabeling for estrogen receptor alpha in normal porcine myometrium (brown = positive). 20X, original magnification.

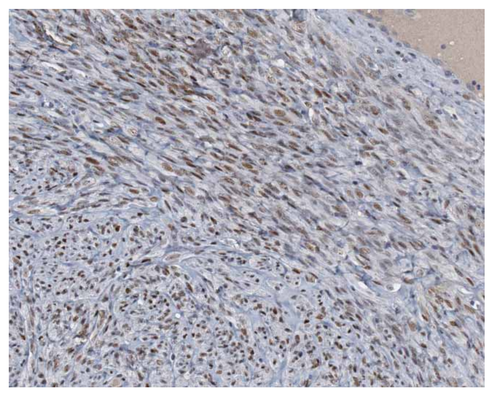

Figure 6. Intense immunolabeling for estrogen receptor alpha in a porcine leiomyoma (brown = positive). 20X, original magnification.

Discussion

Uterine leiomyomas in women are reportedly steroid-dependent, with both estrogen and progesterone receptors immunohistochemically expressed in tumors and matched myometrium. Expression of ER-α in particular has been reported to vary. Some studies report increased expression in tumors, whereas others have demonstrated decreased expression or no change in expression compared to normal myometrium. The majority of studies, however, indicate overexpression of this hormone receptor in leiomyomas versus normal myometrium.

The potbellied pig develops uterine leiomyomas similar to those described in women. The results of this retrospective study indicate that these porcine tumors express ER-α, with variation among individuals as well as between multiple tumors in a given animal. Again, this correlates with the results reported in women. Staining intensity ranged from minimal to intense, but the majority of pigs exhibited elevated steroid hormone receptor expression in the leiomyomas versus matched myometrium, and results were statistically significant (Figure 2).

Studies are underway to assess the expression of other steroid hormone receptors such as progesterone and estrogen receptor-beta in these porcine tumors. In addition, future studies will endeavor to histologically stage the porcine estrous cycle in order to understand the regulation of ER-α expression in normal and neoplastic tissue. These studies will help to further characterize this animal model and its relevance to fibroids in women.

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