Assessment of Uterine Leiomyomas in the Fibroid Growth Study (FGS) using Histological Sampling Methods

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ABSTRACT

Objectives: The objective of the FGS is to investigate why some fibroids grow to become health problems in women while others do not. Using semi-quantitative histological analyses, we will identify molecular, cellular, and pathological characteristics of the leiomyomas with differing growth dynamics to correlate the histopathologic characteristics of fibroid growth.

Methods: Histochemistry was performed on samples of leiomyoma and myometrium for Ki67, Factor VIII, and Masson's Trichrome. Random images of fibroids and myometrium sections were captured with a macro specifically designed for this study and analyzed using Image-Pro Plus image analysis software. The number of images collected varied based on tissue size with 30 images captured for tissues approximately 1.0 cm3 and 15 images for tissues approximately 0.5 cm3 or less. Cell proliferation was assessed by expression of the proliferative nuclear antigen marker Ki67 using colorimetric vector red and methyl green immunohistochemistry. Factor VIII, an endothelial cell marker, was used to determine tumor vascularity. Each vessel was manually traced using the Image-Pro Plus polygon tool, which determined the area of each vessel as well as the total number of vessels present. Masson's Trichrome stain was used to distinguish muscle from fibrous components of the tumors. The color cube-based identification tool provided by Image-Pro Plus automatically determined the percent of fibrous tissue and smooth muscle tissue.

Results/Conclusions: Preliminary data analysis suggests that fibrous and vascular components of tumors vary with size of the leiomyomas.

INTRODUCTION

Uterine leiomyomas or fibroids are the most common type of reproductive tract tumor in women (1). Although benign, these smooth muscle tumors are the primary cause for hysterectomy with symptoms and complications accounting for one-third of all hysterectomies in the United States. The major complications are excessive uterine bleeding, pelvic pain, and infertility (2). Some data suggests that fibroids can also cause pregnancy complications, such as placenta previa and breech presentation (3).

Current research on uterine fibroids has revealed much about the tumors' dependence on hormones to support their development, yet little is known about the characteristics of leiomyoma growth. Histologic descriptions of uterine leiomyoma provide an important foundation for diagnosis and classification, but have yielded limited insights into the pathogenesis. Microscopically, these tumors are characterized by intersecting bundles of smooth muscle cells admixed with as well as separated by abundant amounts of fibrous connective tissue. Typical uterine leiomyoma generally have low rates of mitosis, although some studies suggest rates of cell proliferation can vary within the tumors depending on stage of menstrual cycle (4, 5). Rates of mitosis also can vary depending on the location of the fibroid. For example, submucosal tumors tend to have higher rates of mitosis and more vessels than tumors located elsewhere (6-9). There is also mixed information concerning the extent of vascularity within fibroids with some papers suggesting that fibroids have increased vascularity while other papers suggest there is a decreased vascularity (10-12). Given the low rates of mitosis, inapparent differences in apoptotic rates, and unremarkable findings about the vascularity of these tumors, it remains a puzzle as to what components of the tumor are contributing its growth.

We intend to address the question of growth by analyzing the histological components of uterine leiomyoma in the Fibroid Growth Study in which in-life growth characteristics of the tumor have been defined by MRI before surgery, as well as, size, location and MRI enhancement of the tumors. Immunohistochemistry techniques used to assess the leiomyomas will be used on a quantitative basis. This study will ultimately add to our knowledge of uterine leiomyomas by identifying histologic components that contribute to the growth of these tumors. Results from this study will hopefully lead to more effective therapies for women.

METHODS

Tissue Collection: In accordance with the study protocol, consent was obtained to collect tumor and normal tissue samples, utilizing tissue that would normally be discarded. Prior to surgery, each fibroid was matched to the most recent MRI and a uterine drawing provided by the radiologist to ensure that the collected samples were correlated with the fibroid observed in the MRI. Radiologic descriptions were recorded for each tumor, including: the anatomical position, dimensions, homogeneity of muscle tissue, and estimated percentage of calcification, hemorrhage, necrosis, and cystic change.

For tumors 5 cm or larger, samples were collected from the anterior pole, posterior pole, right side, left side, and center. Each sample consisted of two 5-mm3 pieces of tissue fixed in 10% neutral-buffered formalin and one corresponding 1-cm3 piece of tissue quick frozen in liquid nitrogen. For tumors less than 5 cm, samples were collected from the same five locations to the extent possible without compromising the sample size.

Samples of normal uterine tissue were collected from the areas adjacent to fibroids and from the endometrial, intramural, and serosal layers at a location distant from the fibroids within the uterus. In each case, two 5-mm3 samples were collected and fixed in 10% neutral-buffered formalin and one 1-cm3 sample was quick frozen in liquid nitrogen. Endometrial samples were used to determine menstrual cycle stage.

Histologic Processing: All fibroids and myometrium collected at surgery were processed using the standard H&E technique to confirm basic morphology. For immunohistochemistry, tissue were sectioned at 5 microns, placed on positively charged glass slides and rehydrated.

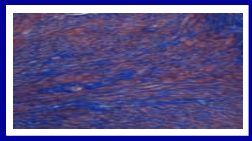
Histologic Tissue Analysis: Random images of fibroids and myometrium were captured with a macro specially designed for this study and analyzed using ImagePro Plus image analysis software (Media Cybernetics, Silver Spring, MD). The number of images collected varied based on tissue size, with 30 images captured for tissues approximately 5-mm3 and 15 images for tissues approximately 0.5-cm3 or less.

RESULTS

Fig. 1 represents the processed images on which percentage of fibrous and smooth muscle tissue was determined using Masson's Trichrome stain (Fig 1a); processed images on which the vessel area (Fig 1b) and cross-section number (Fig 1c) were determined using Factor VIII staining.

Data was summarized (Table 2) and examined statistically to determine whether there were differences in the histological parameters in tumors of different sizes, different locations within the uterus, menstrual cycle stage, or race (Table 1).

Figure 1A: Trichrome Staining



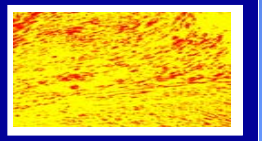


Figure 1B: Factor VIII staining

Figure 1C: Image Analysis from Vessels

Table 1. Determination of what variables effect histology of uterine leiomyoma

Variable	Extent of fibrous tissue within a tumor	Total vessel area within a tumor	Number of vessel cross-sections within a tumor
Effect	P-Value	P-Value	P-Value
Race	0.3647	0.2311	0.0764
Location within uterus	0.0934	0.0129*	<.0001**
Location on uterus	0.5474	0.1352	0.0451
Size	0.0195*	0.0418*	<.0001**
Menstrual cycle Stage	0.9502	0.9295	0.6213

*Significant at p<0.05 **Significant at p<0.001

Table 2. The differences in tumor size were further examined to reveal histological patterns that may be associated with fibroid growth

Pairwise Comparisons*	Trichrome Data	Vessel Area	Vessel Number
Large vs. Medium	p=0.0055* mean=6.9+/-2.5	p=0.0742 mean=0.19+/-0.076	p=0.0016* mean=-6.5+/-0.91
Large vs. Small	p=0.0403* mean=4.9+/-4.9	p=0.0124* mean=0.14+/-0.080	p < 0.0001** mean=-3.0+/-0.96
Medium vs. Small	p=0.3236 mean=-2.1+/-2.1	p=0.4868 mean=0.047+/-0.07	p<0.0001** mean=-3.5+/-0.80

*All comparisons were made with a sample size of at least 300 images

CONCLUSIONS

These data suggest that:

1. Histologic components of uterine leiomyoma examined in this study vary significantly with the size of the tumor and its location. Fibroids greater than 7 cm^3 have a significantly larger fibrous component than smaller tumors. The vascular density also varies with size where larger tumors appear to have a greater area of vessels but less cross-sectional number of vessels.
2. Histological components examined in this study did not differ between races or menstrual cycle stage.

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ACKNOWLEDGEMENTS

This study is co-funded by the National Institute of Environmental Health Sciences (NIEHS) and the National Center for Research on Minority Health and Health Disparities (NCMHD), National Institutes of Health (NIH), Department of Health and Human Services (DHHS).

This study is conducted through the General Clinical Research Center at UNC, Chapel Hill, NC under grant #MO1RR00046.

Support to Integrated Laboratory Systems, Inc is provided under NIEHS Contract Nos. N01-ES-95446 & 273-01-C-0157.

This study is approved under the annual review of IRB 01-E-N209 at the DHHS NIH NIEHS and GCRC-1762 at UNC.