Proceedings from the National Institute of Child Health and Human Development Conference on the Uterine Fibroid Research Update Workshop

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The purpose of the National Institutes of Health conference Fibroid Research Workshop in September 2007 was to bring Eunice Kennedy Shriver National Institute of Child Health and Human Development–funded fibroid investigators together to discuss basic science and clinical research advances on uterine leiomyomata. General topics included advances in epidemiology, etiology, therapeutic approaches, and clinical trial challenges; suggestions for advancement of basic understanding, clinical intervention, clinical trials, and future directions were highlighted. (Fertil Steril® 2011;95:9–12. ©2011 by American Society for Reproductive Medicine.)

Key Words: Leiomyoma, myometrium, epidemiology, etiology, therapy, clinical trials

The Eunice Kennedy Shriver National Institute of Child Health and Human Development sponsored a Uterine Fibroid Research Update Workshop. The focus of this initiative was to review the latest research findings among National Institutes of Health–supported researchers in uterine leiomyomata. Investigators reviewed data on leiomyoma epidemiology, etiology, therapy, and clinical trial challenges. The findings of this conference are summarized in this document, with supporting manuscripts included.

EPIDEMIOLOGY

Racial Disparity and Assisted Reproductive Technologies

In a retrospective study evaluating outcomes of IVF in women with leiomyomata, race was self-identified but independently verified. African American women had higher utilization rates for ART, and their outcomes were worse than those of other races. The spontaneous abortion rate was significantly higher among African American women. Presence of leiomyomata was associated with a reduced likelihood of pregnancy, irrespective of race. African American women had significantly greater numbers of leiomyomata. When comparing racial groups and controlling for the presence of fibroids, no differences in pregnancy outcomes were observed (1).

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Racial Disparity and Genetics

Several studies have demonstrated an increased prevalence of leiomyomata in African American women compared with Caucasian women. African American women were diagnosed earlier (age 31 years vs. 37 years) and were more likely to have severe symptoms (30% vs. 15%). In support of a heritable predisposition to development of leiomyoma, genetic syndromes such as hereditary leiomyomatosis and renal cell cancer demonstrate alterations in specific genes, such as fumarate hydratase. Other identified candidate genes include *HMGA2*, *HMGA1*, *CYP1A1*, *CYPA13*, and *MORF*. Expression of *HMGA2* exhibited greater variation in African American women compared with Caucasian women. Using single nucleotide polymorphism analysis, there was a significant linkage of the C allele of a 3′ untranslated region of *HMGA2* in Caucasian women (2).

Racial Disparity and Catechol-O-Methyltransferase Polymorphisms

Catechol-O-methyltransferase (*COMT*) converts catechol estrogens into methoxy derivatives that have hormonal and metabolic effects. *COMT* is overexpressed in leiomyoma compared with adjacent myometrium. The wild-type *COMT* val/val variant has significant enzymatic activity. The *COMT* val/met polymorphism has intermediate activity, whereas the met/met variant has very low enzymatic activity owing to thermal instability. The high-activity *COMT* val/val polymorphism is associated with increased risk of leiomyoma. The *COMT* val/val genotype is more prevalent in African American women and might be an explanatory factor for their higher risk of

uterine leiomyomata. *COMT* inhibitors might be a potential medical intervention for leiomyomata (3).

Leiomyomata and Miscarriage

A prospective study was performed to investigate the association of leiomyomata with spontaneous abortion for a cohort in which fibroids of 0.5 cm or more were documented early in pregnancy. An ultrasound scan was scheduled as close to 5 to 6 weeks' gestation as possible, with further data obtained at 10–12 weeks and 22–24 weeks to ensure that all pregnancy losses were reported. The presence of fibroids was not independently associated with risk of miscarriage. With the exception of submucous fibroids, there was no evidence that fibroid characteristics increased the risk of miscarriage. However, additional evaluation is required before reaching conclusions about possible effects of smaller, intramural, mucous fibroids on pregnancy (4).

Psychosocial Stress and Leiomyomata

Psychological stress has been associated with mental and physical health outcomes, such as obesity, atherosclerosis, and hypertension—all potential risk factors for leiomyoma development. Stress could increase risk of uterine fibroids through several possible mechanisms, including disruption of the hypothalamic–pituitary–adrenal axis. Data suggested that a link might exist between stress and the number of leiomyoma. Additional analyses using multivariate models are planned. In addition, plans to examine perceived racism as a chronic stressor and as a risk factor for fibroids in African American women are under development. Sensitivity analyses and Bayesian analyses will be conducted to evaluate reverse causation.

ETIOLOGY

Altered Cell Differentiation

Leiomyoma surgical specimens demonstrated reduced expression of gene products involved in retinoic acid (RA) production and increased expression of gene products involved in RA degradation. Furthermore, leiomyoma tissues exhibited more rapid metabolism of RA when the hormone was added exogenously. When RA was added to immortalized leiomyoma cells in tissue culture, expression of genes involved in RA production increased to expression levels found in myometrial cells; conversely, genes involved in RA degradation decreased to expression levels found in myometrial cells. Retinoic acid treatment of immortalized leiomyoma cells altered expression of many genes encoding extracellular matrix (ECM) proteins, and levels of expression resembled expression levels observed in myometrial cells. In contrast, treatment of immortalized myometrial cells with transforming growth factor (TGF)-β3 caused immortalized myometrial cells to develop a leiomyoma-like ECM phenotype (5–10).

Smooth Muscle Hyperproliferation

CCN5 is a secreted matricellular protein that is down-regulated in human leiomyomata. It is unique among the CCN family of proteins because it does not increase proliferation, motility, or expression of matrix metalloproteinases (MMPs). *CCN5* function seems to vary by cell type; in some cells, *CCN5* is a scaffolding protein. *CCN5* knockdown increases uterine smooth muscle cell (SMC) motility by altering the α-actin cytoskeleton. Furthermore, *CCN5* expression was rapidly decreased when SMCs received a signal to proliferate. Collagen types I and II were significantly down-regulated in uterine SMCs when exposed to *CCN5* protein. *CCN5* is an important regulator of SMC function in both normal and diseased states (11).

Progesterone Action

Progestins and antiprogestins have important biologic and therapeutic effects on uterine leiomyoma. Mechanistically, progesterone (P) receptor–ligand complexes interact with promoters of a number of genes and regulate gene expression in leiomyoma cells. The products of these genes reduce apoptosis and enhance proliferation (e.g., *BCL2*, *KLF11*), thereby promoting growth of leiomyomata. Conversely, P antagonists exert therapeutic effects via targeting of these genes and reversal of the mitogenic effects of P. Furthermore, P receptor binds at sites throughout the entire genome at previously unrecognized sites. In addition, effects of progestins on leiomyoma growth potentially include nonclassic signaling through interaction with membrane progestin receptors (12–15).

Aromatase Regulation by Leptin

Leptin is a plausible regulator of aromatase, given the coexpression of leptin and aromatase by adipocytes. Leptin also stimulates collagen production and may therefore play a role in leiomyoma formation. Treatment of primary leiomyoma cells in culture with leptin resulted in increased aromatase expression. Furthermore, leptin treatment resulted in phosphorylation of JAK-2 and STAT3, whereas cotreatment with a JAK-2 phosphorylation inhibitor prevented the leptin-regulated increase in aromatase expression. Chromatin immunoprecipitation analysis demonstrated STAT-3 binding to the aromatase promoter I.4, suggesting a possible mechanism for leptin regulation of aromatase in leiomyomata.

Altered Mechanical Homeostasis

Clinically, leiomyomata are firm tumors. Cells within a leiomyoma grow and proliferate in a milieu of increased mechanical stress. Studies demonstrated that the ECM secreted by leiomyoma cells was disordered and featured altered expression of proteins, such as versican. The increased mechanical stress was accompanied by activation of the small guanosine triphosphate–binding protein Rho, and increased levels of Rho-GEF, AKAP13. Studies have shown that the apoptotic index is reduced in leiomyomata, and activation of Rho has been shown to promote a proliferative phenotype in some cells. Application of mechanical strain to myometrial or leiomyoma cells in culture revealed fundamental differences in levels of Rho activation. Leiomyoma cells may secrete more ECM as a result of the increased mechanical stress, but they do so in a disordered and dysregulated fashion. Whether application of mechanical stress would alter the growth of leiomyoma cells remains to be tested (16).

ECM Regulation

Halofuginone inhibited TGF-β signaling by leiomyoma SMCs. Furthermore, proliferation assays revealed a dose-dependent inhibition of DNA synthesis by halofuginone for both leiomyoma and myometrial cells. Halofuginone reduced mRNA levels of collagen types I and III in leiomyoma and myometrial SMCs. Furthermore, halofuginone-mediated inhibition of myometrial and leiomyoma cell proliferation was reversible when the drug was removed. Adding monomeric bovine collagen type I reduced the inhibitory effect of halofuginone on leiomyoma cell proliferation. It was the inhibition of collagen production that seemed to be responsible for proliferation inhibition. Monomeric collagen stimulated growth of leiomyoma cells (17).