Summary of the Proceedings of the Basic Science of Uterine Fibroids Meeting: New Developments February 28, 2020

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Abstract
Scientists from multiple basic disciplines and an international group of physician-scientists from the field of obstetrics and gynecology presented recent studies and discussed new and evolving theories of uterine fibroid etiology, growth and development at The Basic Science of the Uterine Fibroids meeting, sponsored by the Campion Fund and the National Institute of Environmental Health Sciences. The purpose was to share up-to-date knowledge and to stimulate new concepts regarding the basic molecular biology and pathophysiology of uterine fibroids, and to promote future collaborations. The meeting was held at the National Institutes of Environmental Health Sciences in North Carolina on February 28, 2020. Speakers reviewed recent advances in cellular and molecular processes that contribute to fibroid growth and new opportunities for treatment. At the conclusion of the conference, attendees identified important new directions for future research.

Capsule:
A summary of the presentations on the Basic Science of the Uterine Fibroids.

Introduction
The Campion Fund and the National Institute of Environmental Health Sciences sponsored a meeting on the Basic Science of Uterine Fibroids on February 28, 2020. The purpose of the meeting was to present current research and to stimulate further basic studies through collaboration. Presentations included recent discoveries and theories integrating current and evolving concepts and are summarized in this article. The presentations demonstrated the complexity of fibroid biology. A final session was a discussion that lead to a number of future research questions that are included here.

Because this paper presents material discussed at a meeting, it does not represent all basic science pertaining to uterine fibroids and should not be considered a review of the topic.

FINDINGS OF INVITED SPEAKERS

Plenary Presentation

**Stem cells, epigenome and steroids in uterine leiomyomas**—Uterine leiomyomas or fibroids, comprised of abnormal smooth muscle cells and abundant extracellular matrix, (ECM) are dependent on ovarian steroids for growth and can cause excessive uterine bleeding, anemia and recurrent pregnancy loss. Each leiomyoma originates from clonal expansion of a single smooth muscle progenitor/stem cell, which bears a single distinct mutation (e.g., *MED12* or *HMGA2*). These mutations initiate chromosomal instability or genome-wide epigenetic perturbations. Estrogen together with estrogen receptor-α (ERα) renders leiomyoma responsive to progesterone by induction of PR expression. PR binds to tens of thousands of DNA sites in leiomyoma smooth muscle cells to regulate multiple genes and promote proliferation, survival and abnormal production of extracellular matrix. Treatment of patients with anti-progestins strikingly diminishes the tumor size and associated symptoms. A distinct leiomyoma stem cell population with self-renewing capacity, comprising 5% of the tumor is indispensable for robust tumor formation in
response to estrogen and progesterone in vivo. These stem cells, are ERα or PR-deficient but rich in the cell surface receptors for cytokines and growth factors originating from the surrounding ERα/PR-rich differentiated cells in response to estrogen and progesterone (Figure 1). Dysregulation of DNA methylation in tumor stem cells plays crucial roles in disease progression. Leiomyoma stem cells are uniquely DNA hypermethylated at the PR gene locus and its target regions suppressing stem cell response to progesterone. DNA methylation inhibitor 5’-Aza depletes stem cells via increasing PR expression and its target genes. 5’-Aza treatment significantly reduced the stem cell numbers and their tumor initiation ability (1–4).

**Featured Presentation**

**Mechanosensing and fibrosis**—Mechanosensing describes the ability of cells to respond to changes in their mechanical environment. This occurs through cellular interactions with other cells or the extracellular matrix (ECM). The ECM is deposited during development and maintained throughout life to serve as a scaffold and mechanical support for tissue function. Mesenchymal cells including fibroblasts and smooth muscle cells take a leading role in depositing the ECM and determining its architecture and mechanical properties (5). These cells are also responsive to changes in ECM composition and mechanical environment, generating feedback loops between ECM remodeling and cellular mechanosensing. Such feedback loops can be important in maintaining tissue homeostasis, but can also become corrupted in pathological conditions, as is the case in fibrotic tissue remodeling (6). In such settings, matrices of pathologic stiffness promote profibrotic signaling and enhanced deposition and remodeling of ECM, leading to feedback loops that promote progressive fibrosis (6). There is growing evidence of mechanosensing involved in fibroid growth (7). Ongoing work is identifying the mechanically activated pathways in mesenchymal cells that promote ECM deposition and the disease-related changes in ECM composition and mechanics that regulate cell function. Uncovering the control systems by which cells respond to and regulate the matrix, and the failures in these homeostatic mechanisms that lead to pathological tissue remodeling, remains a major challenge. Ultimately, a detailed understanding of the molecular mechanisms linking the altered mechanical environment to cellular activation in uterine fibroids may enable new approaches to arrest or reverse fibroid growth and pathological ECM remodeling.

**Current Research Contributions to Understanding Fibroid Biology**

**HOXA gene cluster biology in uterine fibroids**—Unsupervised clustering of results from RNA-seq and DNA methylation analyses segregates normal myometria from fibroids, and further segregates the fibroids into subtypes characterized by MED12 mutation or activation of either HMGA2 or HMGA1 expression. Hypomethylation of the HMGA2 gene body is observed in HMGA2 overexpressing fibroids compared to normal myometria and the other fibroid subtypes. Chromatin compartmentalization of HMGA2 locus is inferred from the DNA methylation results and shows open areas only in HMGA2 overexpressing fibroids (8). Additionally, HOXA13, a critical homeobox gene for proper posterior Müllerian duct differentiation, is not normally expressed in the uterus, but is expressed in fibroids (Figure 2 left). The RNA-seq results show a switch to more posterior expression in the HOXA gene cluster in all fibroids. Overexpression of HOXA13 in a myometrial cell line
correlates with altered expression of typical uterine fibroid genes, suggesting that activation of HOXA13 expression could be a driver of the disease (8). HOXA13 is normally expressed in the cervix, and a significant overlap of differentially expressed genes between cervical stroma and uterine fibroids compared to normal myometrial is also observed in the RNA-seq results. These observations suggest that homeotic transformation by chromatin modification in myometrial cells induces the cells to develop a more cervical stroma phenotype (Figure 2 right). Exploring this epigenetically-driven phenotypic change in more granular detail will be important for understanding the etiology and pathobiology of the disease (8).

The life cycle of the uterine fibroid myocyte—Observations of light and electron microscopic, immunohistochemical, and morphometric studies have led to the hypothesis that fibroid changes over time may relate to the excessive production of collagen by phenotypically transformed myocytes. This accumulation of collagen results in decreased micro-vessel density, followed by myocyte injury and atrophy, with eventual senescence and involution through ischemic cellular degeneration and inanition. Uterine leiomyomas, or fibroids, are characterized by two histologic features, proliferation of myocytes and production of an extracellular collagenous matrix. In the larger tumors, the collagenous matrix is often abundant. Within those regions in which the accumulating collagen is excessive, the myocytes are progressively separated from their blood supply, resulting in myocyte atrophy and eventually cell death. It is within these hypocellular, hyalinized areas that the complete lifecycle of the fibroid myocyte occurs (9–11). It begins with the phenotypic transformation of a contractile cell to one characterized by proliferation and collagen synthesis, progresses through an intermediate stage of atrophy related to interstitial ischemia, and ends in cell death due to inanition. Lastly, resorption of inanotic cells appears to occur by a non-phagocytic, presumably enzymatic process of degradation and recycling that is referred to as reclamation (Figure 3).

Environmental phthalates exposures, microRNA expression, and uterine fibroid severity—Phthalates are a class of multi-functional chemicals commonly used in personal care and consumer products (12). Exposures are widespread among reproductive-aged women and can vary by race/ethnicity (12). Evidence from both animal and human studies suggests that exposures to certain phthalates are associated with adverse reproductive outcomes (13). A preliminary, cross-sectional study of 57 pre-menopausal women undergoing a hysterectomy or myomectomy was conducted to evaluate associations between phthalates exposures and measures of fibroid burden (i.e., diameter of largest fibroid and uterine volume) and microRNA expression in fibroid tumors and myometrium (14,15). Most women were Black, overweight or obese, and college-educated. Geometric mean of 3 phthalate metabolites were >30% higher in Black women compared to White or Latina women. In multivariable models, a doubling in the sum of di(2-ethylhexyl) phthalate metabolites (∑DEHP) and the weighted sum of antiandrogenic phthalate metabolites (∑AA Phthalates) was associated with 26.81% (95% CI 2.19, 57.37) and 33.19% (95% CI 6.59, 66.43) increase in uterine volume, respectively (14). In epigenetic analyses of fibroid tumors, mono-hydroxybutyl phthalate and mono-hydroxyhexyl phthalate were positively associated with microRNAs, miR-10a-5p and miR-577, respectively. Eight phthalate-miRNA associations significantly varied by race/ethnicity. The mRNA gene targets of
phthalate-associated miRNAs were significantly associated with multiple fibroid-related processes including angiogenesis, apoptosis, and proliferation of connective tissues (15). Thus, phthalates exposures may be associated with fibroid outcomes and microRNA regulation may be involved in biological pathways linking phthalates to fibroid pathogenesis. Validation of these preliminary findings may provide insight into modifiable risk factors and contribute to novel hypotheses regarding racial/ethnic disparities in fibroids.

The central role of AP-1 in collagen deposition in uterine fibroids—Uterine leiomyoma growth is directly related to the aberrant and excessive extracellular matrix deposition (ECM) consisting of collagen, proteoglycans, and fibronectin, among other proteins. Many indirect ECM regulators (vitamins A and D, targretin, fucoidan, celecoxib, simvastatin, curcumin, ECGC, resveratrol, fasudil, locostatin, berberine, and isoliquiritigenin (16–28), many receptors and cell adhesion molecules (TGFBR, EGFR, IGFR, and integrins (29,30–), and many signaling pathways, including Smads, MAP/MAP-K, WNT/b-catenin, JAK/STAT, PI3K/mTOR, Ras/Raf/MEK/ERK, and RANK/RANK (2,31–37) pathways are involved in the development and growth of leiomyomas. Notably, each of these altered pathways regulates transcription via the AP-1 transcription complex. AP-1, a family of transcription factors consisting of FOS and JUN members, form homo- or heterodimers amongst other AP1 members or via basic leucine zipper motifs with other factors such as the ATF family. The TGFβ response element has an overlapping site for AP-1 and cis-SMAD (serving as an AP-1 binding site) involved in the transactivation of COL1A2. Expression of multiple AP-1 members is altered in leiomyomas. pC-Jun, pC-Fos, JunB, JunD, C-Fos, FRA-1, and FRA-2 concentrations are lower compared to patient-matched myometrial cultures, and FOSB at increased concentration (38). TGF-β3 stimulates production of these ECM components, but this stimulation initially requires AP-1 activation. With AP-1 inhibition, concentrations of COL1A, fibronectin, and versican were decreased. While TGF-β3 treatment alone augmented expression of all evaluated proteins, co-treatment with AP-1 inhibitor blocked TGF-β3 augmentation. Multiple signaling pathways are activated in leiomyomas, which converge on AP-1 signaling, to induce the phenotype of aberrant and excessive ECM (Figure 4).

Single cell RNAseq provides a molecular and cellular cartography of the human myometrium and uterine fibroids—Despite the high prevalence and major negative impact of uterine fibroids on women’s health, knowledge about tumor-initiating cells is scarce (39). Whole tissue studies have provided information on chromosomal and/or genetic alterations (40) as well as the putative presence of uterine fibroids stem cells (41). However, they are limited in providing mechanistic and therapeutic insights due to the undefined intra- and inter-tumor heterogeneity (42). The exponential growth of single cell transcriptomics makes it possible to narrow down anatomy at the single cell level as demonstrated in the Human Cell Atlas (HCA) initiative in order to better understand the cellular hierarchy of uterine fibroids and myometrium. Based on this approach, single cell RNAseq analyses were performed by profiling a total of 5,432 single cells from uterine fibroids (F), fibroid-free matched myometrium (M) and healthy myometrium (hM) from 7 patients. Preliminary results based on dimensional reduction revealed the cellular heterogeneity of these tissues (F, M and hM), consisting of 15 cell types and states.
Specifically, three major lineages of smooth muscle cells, fibroblasts and lymphatic endothelium, were identified and which drastically differ among the tumor (F) and the non-tumor (M/hM) tissues. An overall inflammatory state in F was also observed, manifested as the transcriptomic signature of the immune cells, the abundance of lymphatic vessels, and the interplay between CCL21-CCR7. This reveals a molecular and cellular cartography of the human myometrium and uterine fibroids through specific single cell transcriptomes which may provide molecular targets for less invasive treatment of these benign tumors. In summary, novel human myometrial and uterine fibroid specific cell types and states were identified in order to advance the understanding of myometrial tumorigenesis.

Lessons learned from treatment approaches and developmental exposures mapping fibroid clinical behavior to biologic variables.—Hysterectomy with ovarian preservation is the still the leading treatment for uterine fibroids despite multiple effective alternatives to hysterectomy. Moreover, the significant long-term risk of hysterectomy makes this treatment less than ideal (43)(Figure 5). Hysterectomy remains important because both clinical trials and in vitro experiments show there is always unexplained heterogeneity of response among fibroids. While the NIEHS Fibroid Growth Study demonstrates that clinical characteristics including age and race are associated with increased growth, we have no growth-directed or regression triggering therapies. Clinically there are unmeasured variables such as adenomyosis and endometriosis and individual and culturally-influenced responses to specific symptoms. Nonetheless, biologic heterogeneity also likely places a role. There are several barriers to fibroid research. First, although fibroids are clonal, multiple independent clones do arise within the same uterus. Moreover, although we commonly map symptoms to specific fibroids, this has limited precision. Even genetic studies have demonstrated that genotype does not predict phenotype, but only show correlations and thus for most women is not clinically useful (44). Finally, the lack of understanding of the natural history of uterine fibroids handicaps our current clinical care. A longitudinal “Framingham Fibroid Project” would be useful. The Framingham Heart Study was started in 1948 it was almost a decade later that hypertension and hypercholesterolemia were found to increase heart disease and nearly two decades before exercise and obesity were linked to heart disease. Prospective registries such as COMPARE-UF and CAPTURE have been established in the past decade but have not been associated with longstanding support.

Biology and histology of the pseudo-capsule—A growing fibroid causes progressive formation of a peripheral anatomical structure, the pseudo-capsule. This structure originates from the compression of the surrounding myometrium by the fibroid and separates it from the healthy myometrium (Figure 6). The pseudo-capsule shifts the myometrial muscles outward away from the fibroid maintaining the integrity and contractility of uterine musculature. The fibroid is structurally anchored to its pseudo-capsule by connective bridges, but lacks a true vascular pedicle. Occasionally, bridges of collagen fibers and vessels anchoring the fibroid to myometrium interrupt the pseudo-capsule surface. These physio-pathological phenomena result in the formation of a clear cleavage plane either between myoma and the pseudo-capsule, or between the pseudo-capsule and the surrounding myometrium. The pseudo-capsule has the same biological
structure as the myometrium, as visualized by transmission electron microscopy. The pseudo-capsule cells are genetically identical and have the same features of myometrial smooth muscle cells. The pseudo-capsule is a neurovascular bundle containing numerous collagen fibers, neuro-fibers and blood vessels (45). Studies of growth factors show intense angiogenesis in pseudo-capsule vessels. Angiogenetic factors identified in the pseudo-capsule vessels are widely involved in myometrial physiology. These substances have a pivotal role in wound healing and muscular innervation. Neuropeptides and neurotransmitters detected in myoma pseudo-capsule play a role in mediating inflammation and wound healing and in regenerative processes associated with pseudo-capsule sparing during myomectomy, and induce angiogenesis peripherally into myometrium. Post-myomectomy muscle repair processes are important role in uterine physiology, in subsequent pregnancy and in the prevention of uterine rupture (46–48).

Prevention of uterine rupture is a debated topic and uterine scar physiology after intracapsular myomectomy with pseudo-capsule sparing is one of the most important hypotheses to study and test in Ob/Gyn surgery.

**Vitamin D and green tea extract: natural compounds for the treatment of uterine fibroids.**—Uterine fibroids (UFs) remain a significant health issue for many women, with a disproportionate impact on Black women. Unfortunately, the mainstay of treatment is still surgical. As new pharmaceutical medical options emerge, significant limiting factors appear including hot flashes, bone density loss, unfavorable endometrial changes and most importantly, the inability to attempt conception during treatment. This latter challenge is particularly relevant as UFs prevalence peaks during women’s most fertile years. Natural compounds are promising candidates as effective and safe treatments for UFs. Notably, such natural compounds are very well-tolerated with favorable safety profile and are fertility friendly. Examples including vitamin D and green tea extract. Vitamin D wields anti-proliferative power through UFs cell growth arrest, apoptosis induction, and inhibition of extra cellular matrix (ECM) related markers (49). Moreover, vitamin D inhibits the Wnt/β-catenin pathway which is activated in UFs with MED12 mutation compared with normal adjacent myometrium, thereby decreasing fibroid growth (50) (Figure 7). Additionally, vitamin D3 effectively suppressed UF growth in Eker rat animal models (51). Green tea extract (EGCG) inhibits cell proliferation and induced apoptosis in both human UF and rat ELT-3 UF cells in vitro and in vivo animal models, respectively. It markedly reduced tumor volume and weight of female mice after 4- and 8-weeks treatment (53). Moreover, in a double-blinded placebo-controlled randomized clinical trial, 800mg/day EGCG resulted in a significant reduction in UF volume and fibroid-specific symptom severity as well as significant improvement in health-related quality of life. No adverse effects or endometrial hyperplasia were observed (54).

**Heterogeneity of interstitial collagen and stiffness in uterine fibroids**—Uterine fibroids are stiff tumors with abundant extracellular matrix (ECM), including large amounts of collagen (9,10,11,55–57 58–63). Evaluation of 19 fibroids (3–11 cm) from eight women, revealed substantial differences in gross morphology (Figure 8). Classical whorled patterns were noted in only 8 fibroids. Other patterns are described as nodular (n=9), interweaving
Some fibroids (n=6) had characteristics of two or more patterns. Fibroids contained 37–77% collagen (Masson trichrome). Interstitial collagen types, determined in 5 additional samples (serial extraction and gel electrophoresis), included type I, III, and V collagen of different proportions. Fibroid stiffness measured by rheometry revealed considerable heterogeneity. Stiffness among fibroids ranged from 3028 to 14180 Pa (CV 36.7%; p<0.001, one-way ANOVA). Stiffness was not correlated with overall collagen content indicating that collagen crosslinks and other ECM components are important factors. Neither stiffness nor collagen content was correlated with fibroid size. Stiffness within individual fibroids was also not uniform and variability ranged from CV 1.6 to 42.9%. The observed heterogeneity in structure, collagen content, and stiffness highlights that fibroid regions differ in architectural status (58). Therefore, the design of studies exploring the pathobiology of uterine fibroids should account for their heterogeneity because samples from different regions have different characteristics. These differences might be associated with variations in local pressure, biomechanical signaling (59,60), and altered growth. The understanding of fibroid pathophysiology will greatly increase through the investigation of the complexity of the chemical and biochemical signaling and mechanical forces in fibroid development.

**Injectable collagenase as a treatment for uterine fibroids**—Uterine fibroids are a common pathologic fibrotic process characterized by excessive deposition of collagen, especially collagens types I and III (11,61,62). Collagens contribute to the stiffness of the extracellular matrix of uterine fibroids, (63) and in turn, the increased extracellular matrix stiffness contributes to uterine fibroid growth (64). Other pathological fibrotic diseases, such as Dupuytren’s contracture and Peyronie’s disease, have been successfully treated with injection of purified collagenase *Clostridium histolyticum* (EN3835) (65–70). Since studies indicated that collagenase injection digested ex-vivo-treated fibroids (71,72), this clinical trial investigated injectable collagenase as a possible treatment for uterine fibroids using transvaginal delivery of collagenase. The clinical trial was registered (NCT02889848).

Uterine fibroids in twelve women planning a hysterectomy were injected with collagenase. No significant adverse events were reported. Three subjects received a constant dose of EN3835 (Group 1, n=3), followed by surgery at 24–48 hrs. The nine remaining subjects had fibroids injected with collagenase in stepped, increasing dosages (Group 2, n=3 per dose). Comparison of collagenase-treated and control fibroid tissues showed a significant reduction in collagen using several approaches, including picrosirius stains and electron microscopy. Examination showed that even at the lowest doses of collagenase, there was a significant reduction in collagen content following collagenase injection, but at higher doses at 60 days duration there was also a reduction in markers of cell proliferation and growth. Though additional studies are needed, these results suggest that injection of collagenase may lead to a reduction in fibroid growth. These data support further clinical investigation of EN3835 as a potential non hormonal, minimally invasive treatment of uterine fibroids.

**Multiple clinical characteristics separate MED12-mutation-positive and negative uterine leiomyomas**—Findings reported in the referenced paper were not presented but were due to the fact that the speaker was unable to attend the meeting. They were published in the program booklet. *MED12* mutations are significantly associated with
smaller tumor size, conventional histology and subserous location. The number of MED12-positive fibroids is inversely related to parity while the number of mutation-negative tumors is positively associated with a history of pelvic inflammatory disease (73).

PRESENTATIONS OF SUBMITTED ON-GOING RESEARCH

Short Talks

Phenotypic screening for anti-fibrotic and anti-proliferative compounds in primary human fibroid and myometrial cells.—Of 40 compounds tested at a concentration of 3 μM, 14 reduced fibronectin deposition in UF by at least 10%, respectively. At 3 μM, Nintedanib (a multi-kinase inhibitor and known anti-fibrotic) reduced fibronectin deposition most preferentially (19% more) in UF relative to MYO. Our results demonstrate that phenotypic screening in matched UF and MYO pairs can reveal compounds with UF-preferential anti-fibrotic and anti-proliferative effects.

Benign leiomyoma and malignant leiomyoscaroma can be discriminated by combining radiogenomics and molecular diagnostic tools.—Integrated genomic, transcriptomic and imaging metrics, provide a clinical fingerprinting of leiomyoma and leiomyosarcoma which allows to differentiate and further classify these myometrial tumors.

A novel strategy to identify new markers of myometrial stem cells for understanding the pathogenesis of uterine fibroids.—Live cells from non-fibroid myometrial samples were collected from Caucasian women and used for single-cell RNA-seq and identification of myometrial cell subpopulations. When combined with standard RNA-seq results from myometrial cells selected with a current stem cell marker, SUSD2, these results will help us identify new markers for enriching myometrial stem/progenitor cells and study the etiology of uterine fibroids.

Poster Presentations Titles

Natural compound methyl jasmonate shows promising anti-fibrotic effects via inhibition of EZH2 mediated Wnt/β-catenin signaling pathway in human uterine fibroids.

Involvement of DNA damage repair sensor in ethnic disparities of uterine fibroids (abnormal DNA repair damage pathway is abnormal in fibroid tissues of African-Americans compared to Caucasian women).

Chromatin conformation in uterine fibroids (through CTCF binding which controls chromatin conformation).

Up-regulation of pro-inflammatory signaling of stem cells associated with the phenotype of leiomyoma (compared to adjacent myometrium).

Targeted CpG methylation (in HMG2 gene body in uterine fibroid cell line Collection of fibroid tissue from a well-described prospective cohort.

The active phytochemical of cruciferous vegetables, sulforaphane, reduces proliferation and inflammation in uterine fibroid cells.
Prevalence of Vit. D deficiency among premenopausal Kenyan women suffering from symptomatic uterine fibroids versus matched healthy controls.

Localized uterine fibroid therapy injectable thermo-responsive hydrogel degradation study (with significantly decreased extent of tissue elasticity with collagen in hydrogel versus collagenase alone)

Genome-scale identification of reprogrammed genes contributing to a hyper-estrogenized phenotype in the development of uterine fibroids (indicating epigenetic changes in developing myometrium is a risk for fibroid development in later life).

**Future Research Approaches and Questions**

- Perform a morphometric analysis of the neural networks of the pseudo-capsule.
- Leiomyosarcoma lacks a pseudo-capsule, at a molecular level, how does this explain the differences between a leiomyosarcoma and a leiomyoma (fibroid)?
- How do growth factors from the pseudo-capsule stimulate fibroid growth?
- How does the epigenetic landscape for myometrial cells change to allow fibroid development and growth?
- What molecular mechanisms are disrupted by MED12 mutation and HMG A2 over-expression?
- How does homeotic transformation of myometrial cells into a more cervical stroma phenotype occur?
- Can the field characterize not only fibroid pathology, but other agreed-upon fibroid disease classification (such as extent of disease)?
- Can the field identify and further characterize the secondary prevention nutritional supplements?
- Can we identify novel therapeutic pathways aside from pathways that regulate gonadal hormone exposure?
- What is the role of mechanotransduction transcription factors, such as YAP/TAZ in uterine fibroid development and growth? How can mechanosensing mechanisms be integrated into the genetic, hormonal and growth factor and cytokine regulation of uterine fibroid etiology, growth and development?
- Longitudinal studies over decades, similar to the Framingham Study of heart disease, need to be designed and conducted.
- How will we enable the development of non-surgical treatments? Is there a role for a therapeutic agent or multiple drugs injected locally into a fibroid?
- How can leiomyosarcoma vs leiomyoma be diagnosed prior to treatment?
- Is there a role of environmental impacts/exposures/influences and epigenetic events on the pathogenesis of fibroids?
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Figure 1: Fibroid Development from Stem Cells:
Myometrial stem cells undergo mutation to become CD34++, CD49b+ leiomyoma (fibroid) stem cells (LSC). In response to cytokines and growth factors in the surrounding ECM the cells undergo epigenetic changes at the PR locus that decreases PR expression and suppresses response to P4 allowing progression through CD34+, CD49b- leiomyoma (fibroid) intermediary cells (LIC) to CD34-,CD49b- leiomyoma (fibroid) differentiated cells (LDC).
Figure 2: HOXA13 Gene Expression in Fibroid Tissue

A: Gene expression patterns for the HOXA gene family in fibroid samples, MED12 mt (n=14), HMG2A hi (n=4), HMG2A hi (n=2) subtypes, normal myometria (n=15) and in cervical stromata (n=6). Batch information is colored by discovery or validation set. B: Boxplots (boxes, 25%−75%; whiskers, 10%−90%; lines, median) of HOXA13 gene expression in fibroid subtypes in A. These results suggest a homeotic transformation of normal myometrium to cervical stroma-like tissue in fibroid etiology, probably through subtype-independent upregulation of HOXA13 expression. Image taken from (8).
Figure 3: Life Cycle of the Fibroid Myocyte:
The phases of the cycle include proliferation and synthesis of collagen, fibroid myocyte atrophy due to decreased microvascular density and interstitial ischemia leading to cell death by inanosis, followed by reclamation. Illustration by David Sabio. Image taken from (11)
Figure 4:  
A Model: Signaling Pathways in Uterine Fibroids Regulating Transcription via AP-1 Transcription Complex
Figure 5: Weighing the Risks of Hysterectomy with Ovarian Conservation:
The risks are greater than the benefits
Figure 6: A Pseudocapsule Separates a Uterine Fibroid from Surrounding Myometrium

A: Histological photomicrograph of pseudocapsule with schematic drawing of the vascular and neuro fiber network. The pseudocapsule separates the fibroid from the myometrium from which it is derived. B and C: Surgical images showing fibroids surrounded by the pseudocapsule separating it from the myometrium. Image based on information in (45)
Figure 7: *In-Vitro* and *In-Vivo* effects of Vitamin D and EGCG in Uterine Fibroids

VitD: Vitamin D3ECM: extracellular matrix related markers, ER: estrogen receptor, PR: progesterone receptor, EGCG: epigallocatechin gallate, PCNA: proliferating cell nuclear antigen, TGF: transforming growth factor, BMP: bone morphogenetic protein, QOL: quality of life.
Figure 8: Representative Photographs of Tissue Slices Showing Differences in Gross Appearance of Fibroids.

A: Classical irregular whorled pattern; B, C, D: Patterns of nodules; E, F: Trabecular structures; G: Characteristics of multiple patterns. This example shows a trabecular/nodular pattern; H: Not categorized. This example shows a tightly gyrated pattern. I: Myometrial tissue shown for comparison. Note the seedling fibroid embedded in the tissue (white appearance). Ruler (cm) shown for size. Image taken from (57).