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A new hypothesis about the origin of uterine fibroids based on gene expression profiling with microarrays

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This article will discuss some recent insights based on our microarray studies that have emphasized the role the extracellular matrix, transforming growth factor beta, and collagen structure in fibroid formation. These studies led to appreciation of molecular similarities between fibroids and keloids. Collectively, these observations suggest a model of fibroid development based on an abnormal response to tissue repair, resulting in disordered healing and formation of an altered extracellular matrix.

Fibroids are benign growths, and based on cytogenetic studies the tumors within a single uterus are clonal, each arising from a different myometrial cell.[1] Epidemiologic, clinical, and experimental data suggest sex steroids promote growth of the tumors.[2] Increased parity may reduce the incidence of the problem, possibly caused by exposure to progesterone.[3] A genetic predisposition to the condition appears to be present, because a familial association has been shown, and rare genetic conditions, such as hereditary leiomyomatosis and renal cell cancer (HLRCC) feature fibroid development.[4] However, with the exception of a guinea pig model,[5] the Eker rat,[6] and a recently reported transgenic mouse,[7] there are few model systems and the origin of these common tumors remains unknown (for review, see Walker and Stewart).[8]

Although the origin of fibroids remains unknown, as health care providers for women, gynecologists must be keenly interested in defining the cause because such an understanding often leads to successful treatment. As we consider what might cause fibroids, there are some puzzling questions to be addressed such as: Why are fibroids so common? As a neoplasm in prevalence and expense they eclipse all others,[8] but because genomic instability is a hallmark of neoplasia, why do only 40% of fibroids exhibit genomic instability? Also, why are there differences in the prevalence of the disease in black women? Fibroids are 3 times more likely to affect women of African-American ethnicity.[9,10] This last point is illustrated in work by Dr Myers (Figure 1). The increased incidence can be seen in hysterectomy

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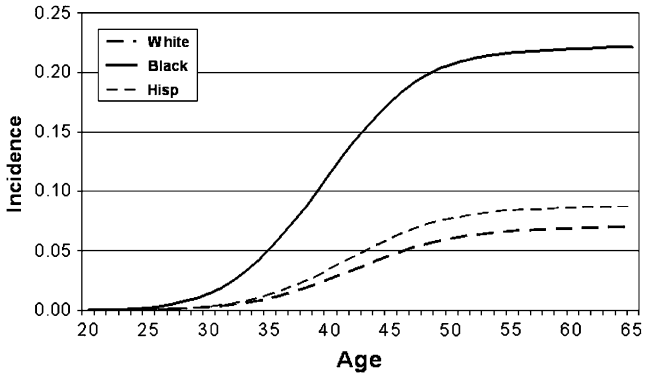

Figure 1 Cumulative incidence of hysterectomy, by race. *Y*-axis: Incidence of hysterectomy. *X*-axis: Age at time of surgery. Data are from analysis of surgical outcomes by Dr Evan Myers (modified and reprinted from with permission "Management of Uterine Fibroids," AHRQ Publication No 01-E052).

rates, which are 3-fold higher in black women, with lifetime risk of hysterectomy approaching 22%. It is logical to assume that a comprehensive explanation for fibroid development must provide an answer to the question: Why is there such a difference in the racial prevalence and incidence of the disease?

Given the apparent clonal nature of fibroids, our group reasoned that the myofibroblast cells comprising fibroids may provide clues to fibroid development. Myofibroblasts are cells of an intermediate phenotype, not quite normal uterine muscle, but neither are they differentiated fibroblasts.[11] Myofibroblasts secrete collagens and other components of the extracellular matrix, but inappropriate function of myofibroblasts has been shown to cause fibrosis.[12] For this reason, our laboratories have focused on gene profiling experiments of these cells.[13,14] Gene profiling, or microarray experiments, enables normal myometrium to be compared with fibroid tumors. The microarray takes advantage of robotics and the information from the human genome project. Few assumptions are required and with high-density genome wide chips available, the approach is almost devoid of inherent bias. Fibroids are particularly well suited to this approach, as the tumors are clonal and normal myometrium from the same patient is available as a control. Understanding the expression pattern would then allow more complex and specific hypotheses to be generated. We[13-15] and others[16-21] have used this potentially powerful approach to study uterine fibroids.

We used oligonucleotide-based chips, specifically Affymetrix HG-U133 A&B chips (Affymetrix, Santa Clara, CA), that contain products from up to 33,000 genes. Fifteen micrograms of total RNA from matched samples from myometrium and leiomyoma were used to generate biotin-labeled complementary RNA (cRNA). For this platform, each sample is prepared separately and hybridized to the chip, then the matched chips are compared by using a computer for the data that have been digitalized. Arrays were analyzed on a Hewlett-Packard Genearray scanner (Hewlett Packard, Palo Alto, CA) using the GeneChip software (Affymetrix). The GeneChip software assigned intensity files for each transcript based on the signal intensity across the 11 pairs of 25 mer oligonucleotide probes of perfect match (PM) or mismatch (MM) sequences. A 1-sided Wilcoxon signed rank test was used to assign a detection *P*-value. After background subtraction based on 1-step Tukey's biweight estimate of transcript expression, global scaling (using 500 as target intensity) was used to normalize and control for any differences in probe intensities. Candidate genes were eliminated if their signal intensity was below 250 U, based on a scatter plot. For pair wise comparison for differences in expression, a Wilcoxon signed rank test generated *P*-value "change calls" of fold changes in transcript expression of either up/increase (+) or down/decrease (−). We used a cutoff of more than 2.0-fold for further investigation of gene expression. The data presented in this article were based on 4 pair wise experimental-control comparisons with an average-fold change across the experiments. In addition, differences in gene expression were confirmed by using other approaches, such as reverse transcription polymerase chain reaction (RT-PCR), real-time PCR, and for some gene products confirmed the differences in protein expression by immunohistochemistry.

Our first observation was that there were some differences between arrays from different core facilities and different Affymetrix platforms. For instance, using HG-U133 chips and a different core, we found differences between our collaborator and our results. Furthermore, if we simply performed an array on 1 sample, and repeated the array a second time, we observed differences in levels of gene expression.[22] This is not too surprising given the vast number of genes sampled. We interpret the differences to be largely because of variation in procedure, subtle differences in hybridization, RNA handling and probe preparation, and data management. To address this concern (gene-specific reproducibility) we repeated the microarray experiments across several specimens and focused our attention on genes identified to be differentially regulated across the experiments.[14] Stated differently, a single experimental comparison is not as meaningful as repeated observations across several experiments. However experiments are performed, at the single gene level of accuracy, microarray results must be confirmed using an ancillary approach to quantify amounts of RNA present, especially in the instance of uterine fibroids.

The second somewhat surprising result was that genes involved in sex steroid action were not featured as differentially expressed genes. For instance, estrogen receptor (ER) alpha, ER-β, progesterone receptor, and nuclear cofactors such as steroid receptor cofactor 1 (SRC-1) and p300/CBP were not different in fibroids