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Insights from gene arrays on the development and growth regulation of uterine leiomyomata

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Abstract

Objective—To use microarray analysis as an unbiased approach to identify genes involved in the induction and growth of uterine leiomyomata.

Design—Screen by arrays for up to 12,000 genes in leiomyoma (L) and control myometrium (M) from nine patients.

Setting—University research laboratories.

Patient(s)—Nine patients in the follicular and luteal phases of the menstrual cycle.

Intervention(s)—mRNA from L and M was converted to biotin-labeled cRNA and hybridized to cDNA oligonucleotide sequences on the arrays.

Main Outcome Measure(s)—Greater than two-fold change in gene expression between leiomyoma and matched myometrium.

Result(s)—Prominent among the 67 genes overexpressed in L relative to M were dlk or Pref-1, doublecortin, JM27, ionotropic glutamate receptor subunit 2, apolipoprotein E3, IGF2, semaphorin F, myelin proteolipid protein, MEST, frizzled, CRABP II, stromelysin-3, and TGFβ3. The genes dlk, IGF2, and MEST are paternally expressed imprinted genes, and the others are involved in tissue differentiation and growth. Prominent among the 78 genes down-regulated in L relative to

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M were alcohol dehydrogenases 1α–γ, tryptase, dermatopontin, thrombospondin, coxsackievirus receptor, nur77, and c-kit.

Conclusion(s)—Arrays offer large-scale screening of mRNA expression, which will help us differentiate between the genes and metabolic pathways necessary for leiomyoma growth and those regulating myometrial contractions.

Keywords

c-kit; dlk; genomic imprinting; mast cells; myometrium; PPARγ; retinoic acid; RNA editing; RXRα; stem cell factor

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Uterine leiomyomata are benign, monoclonal, smooth muscle cell tumors that may be found in up to 70% of reproductive age women (1). Leiomyomata represent a significant public health problem because of their prevalence and associated problems of pelvic pressure, uterine bleeding, pain, and infertility. It is estimated that 25% of women in the third to fourth decades of life seek care for leiomyoma-related symptoms. Despite their common clinical occurrence, the cause of leiomyomata remains obscure.

It is clear that leiomyoma growth is estrogen dependent and P-related since tumors regress after treatment with [1] GnRH agonists (2), which inhibit leiomyoma aromatase (3) and induce a hypoestrogenic state; [2] raloxifene (4); and [3] RU486 (5). Cytogenetic studies have revealed that leiomyomas are monoclonal and specific chromosomal regions may be abnormal in up to 40% of tumors, specifically chromosomes 6, 7, 12, and 14 (6). It is intriguing that 60% of leiomyomata do not exhibit a cytogenetic abnormality. Similarly, detailed studies of syndromes of leiomyoma development [(7) and references therein] have not elucidated the etiology of leiomyoma formation in common cases.

The comparison of leiomyomata to cancer is tenuous since leiomyomata rarely progress to malignancy and the absence of specific chromosomal anomalies common to a leiomyoma and leiomyosarcoma suggest independent pathways of development (8). This fact suggests that an event(s) may trigger abnormal but noncancerous growth within the myometrium.

In agreement with this notion, it was reported that estrogens and all-trans retinoic acid could induce uterine leiomyomata in a guinea pig model (9). Similarities between this model and spontaneous human leiomyomata suggested new mechanism involving estrogens (aromatase), all-trans retinoic acid, and nuclear receptors PPARγ and RXRα. This mechanism supports the idea that leiomyomata may more closely resemble an abnormality of differentiation rather than oncogenesis.

As an unbiased approach to identify genes involved in leiomyomata development, we used gene arrays (10) to simultaneously compare the expression of up to 12,000 genes in leiomyomata and tumor-free myometrium. Among solid tumors, uterine leiomyomata are less complex, being benign, monoclonal, anatomically well defined, and surgically accessible.

Given the high molecular “resolution” of the array technology, and the hormonal changes that occur during the menstrual cycle, we sampled patients in both the follicular and luteal

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phase of the menstrual cycle. Array analysis revealed that 145 genes, most of which were not previously associated with leiomyoma formation, were either up-regulated or down-regulated by more than two-fold in leiomyomata compared with control myometrium. Many genes in this study and that by Li et al. (11) are common to PPARγ/RXRα pathways, neurological conditions, developmental pathways, and the contraction apparatus of the myometrium.

MATERIALS AND METHODS

Uterine tissues were obtained from nine women according to procedures approved by the Institutional Review Board of the University of South Florida. Endometrial dating and the patient's history determined the phase of the cycle; none of the patients received any hormonal medication 3 months before hysterectomy.

Tissue samples were taken within 20–30 minutes of extirpation of the uterus and stored at −75°C. One leiomyoma from each uterus was selected that was white in appearance, larger than 2 cm in the shortest dimension, and from a region near the capsule of the leiomyoma, as shown in Figure 1. We excluded the serosa and grossly necrotic, infracted, or calcified segments of leiomyomata. Tumor-free myometrium was removed at a distance (≥1 cm) from the endometrium, preferably in the miduterine area, unless it was close to another leiomyoma. In the latter case, myometrium was sampled from a more remote area but not an area that involved the cervix.

The anatomical position of the tissues collected was recorded for future reference, since gradients in the expression of certain genes in myometrium may occur along the uterine axes and vary with the phase of the cycle, as was shown with endometrial estrogen and P receptors (12).

Five uteri were in the follicular phase of the cycle on the day of hysterectomy and four in the luteal. Patients from both phases of the cycle were similar in terms of age and body mass index (BMI), except for patient 8 (Fig. 1). Race and increased BMI are associated with the incidence of leiomyomata (13, 14).

Each gene on the array is represented by 16–20 complimentary 25-mer nucleotides (probes), the perfect match, from different parts of the gene but mostly from the 3′ end. On the array, there are mismatch control oligonucleotides that differ from their perfect match partners at a single base, the 13th out of 25 positions.

Preparation of Labeled cRNA Targets for Hybridization

Preparation of cRNA and subsequent steps leading to hybridization, scanning, and data analysis were according to Affymetrix guidelines (Affymetrix, Santa Clara, CA) (15). Briefly, RNA was isolated from 0.5 g of leiomyoma and matched myometrium with the TRIzol Reagent (Invitrogen, Carlsbad, CA) and further purified by the RNeasy procedure (Qiagen, Valencia, CA). RNA quality was assessed by agarose electrophoresis and absorbance at A_{260}/A_{280}.

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Double-stranded cDNA was synthesized from 10 μg total RNA by the Superscript Choice System (Invitrogen) containing a HPLC-purified oligo$(dT)_{24}$ primer (Genset, La Jolla, CA) and T7 RNA polymerase promoter sequence at its 5′ end (5′-GGCCAGTGAATTGTAATACGACTCAC-TATAGGGAGGCGG-$(dT)_{24}$-3′). The second cDNA strand was synthesized using E. Coli DNA polymerase I, RNase H, and DNA ligase. Reactions mixtures were extracted with phenol/chloroform/isoamyl alcohol (Ambion, Austin, TX), and cDNA was precipitated with ethanol and resuspended in RNase-free water.

Labeled cRNA was generated from cDNA by an in vitro transcription reaction with a bioarray high-yield RNA transcript labeling kit (Enzo Diagnostics, Farmingdale, NY) to incorporate biotinylated CTP and UTP. Biotin-labeled cRNA was purified with an RNeasy column (Qiagen) and degraded to 35–200 base fragments at 94°C for 35 minutes in 40 mM Tris-acetate, pH 8.1, 100 mM potassium acetate, and 30 mM magnesium acetate. The integrity of cDNA, cRNA, and fragmented cRNA was assessed by agarose electrophoresis.

Array Hybridization

The hybridization buffer contained 100 mM MES, 1 M NaCl, 20 mM EDTA, 0.01% Tween 20, 0.5 mg/mL acety-lated BSA (Invitrogen), and four control bacterial and phage cRNA (1.5 pM BioB, 5 pM BIO C, 25 pM BioD, and 100 pM Cre) to establish hybridization efficiency. The hybridization buffer was used to prehybridize the test-3 gene array for 10–15 minutes at 45°C. A test array determines target quality and labeling efficiency before using the GeneChip HuGeneFL6800 (uteri 1–4) or U95A arrays (uteri 5–9). The prehybridized solution was removed and replaced with 200 μL hybridization mixture containing hybridization buffer, fragmented cRNA (0.05 μg/μL), and herring sperm DNA (0.1 mg/mL; Promega, Madison, WI). The arrays were hybridized for 16 hours at 45°C, washed with the Affymetrix fluidics solution, stained with 10 μg/mL streptavidin-phycoerythrin (Molecular Probes, Eugene, OR), and scanned with the Hewlett-Packard GeneArray Scanner (Palo Alto, CA) (16).

Data Analysis

Scanned images were visually inspected for hybridization artifacts and analyzed by the Affymetrix Microarray 4.0 software. Arrays were scaled to an average intensity of 1,500 and analyzed independently. To select the important genes in each uterus, we arbitrarily chose (17) a threshold value of >+ 2 or <−2 in the fold change between leiomyoma and myometrium calculated by Affymetrix software.

RESULTS

General

Surgical specimens from nine women were studied (Fig. 1, Tables 1 and 2). Complimentary RNA was prepared from leiomyoma and control myometrial tissue from each patient's uterus. In both Tables 1 and 2, results for a few genes are not listed in the L1:M1 to L4:M4 columns because these samples were tested with the HuGeneFL6800 arrays that contained 6,800 genes per array (chip), compared with 12,000 genes in the U95A arrays (L5:M5 to L9:M9).

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Among the 16–20 probes on the array for each gene, all probes do not hybridize equally well, but among the nine uterine pairs, the same probes show intense hybridization as, for example, for dlk and the myelin proteolipid protein. It is important that without exception positive or negative array ratios L:M agreed qualitatively with those reported in the literature or from our Western blots (data not shown) for IGF1, IGF2, IGFBP-5, stromelysin-3, frizzled, CRABPII, TGFβ3, PPARγ, tryptase, c-fos, cyr61, and c-kit.

The results are sorted in Tables 1 and 2 by the mean ratio L:M among the nine uteri in a descending and ascending order, respectively; this scoring system cannot assure stability of the results because of the limited number of samples. Genes can be searched by name or accession number at the National Library of Medicine Web site http://www4.ncbi.nlm.nih.gov/entrez/query.fcgi. A new Affymetrix site offers complete documentation on genes and probes (http://www.affymetrix.com/index.affx).

Consistent findings were observed among the nine women studied, suggesting that the gene products reported in Tables 1 and 2 represent essential or obligatory growth elements common to the majority of uterine leiomyomata tested rather than idiosyncratic variations present in one uterus. We excluded genes with large positive to negative fluctuations in fold change among the nine uteri as, for example, in the high-mobility group proteins (HMGIC, HMGIY) (18, 19) or SOX20. Nonetheless, 4% of the entries in Tables 1 and 2 show small variations among the nine uteri <+2 or > −2, our cutoff values. The software was unable to calculate accurate fold change in 15%–20% of entries, and genes shown in Tables 1 and 2 with a similarity sign (~) reflect this approximation.

Although only 12,000 of all currently known cDNAs were represented on the arrays, for example, RXRα probes were not represented, we have found some very interesting genes that were consistently up-regulated (Table 1) or down-regulated (Table 2) in leiomyomata.

Genes Up-Regulated in Leiomyomata

Table 1 shows 53 genes, those most highly expressed among 67 genes up-regulated by more than two-fold in L than M in the nine patients (L1:M1 to L9:M9).

Up-regulated genes of special interest include paternally expressed imprinted genes dlk, IGF2, and MEST (20) (see Discussion section), doublecortin, JM27, ionotropic glutamate receptor subunit 2, apolipoprotein E3, IGF2, semaphorin F, myelin proteolipid protein, MEST, frizzled, CRABP II, stromelysin-3, and TGFβ3. Upregulation of TGFβ3 is consistent with a low mitogen cellular environment seen in leiomyomata. Frizzled, the Wnt receptor, and N-cadherin were also up-regulated. The Wnt/frizzled signaling pathway has been shown to influence smooth muscle differentiation acting through a cadherin-dependent pathway (21).

IGFBP-5 was overexpressed in leiomyomata (Table 1), although at the protein level IGFBP-5, may be regulated by pregnancy-associated plasma protein A (22), a protease that was consistently overexpressed in leiomyomata (mean L:M = 1.5). Li et al. (11) used Affymetrix arrays Hu-GeneFL6800 and found that the following genes were up-regulated in leiomyomata: IGF2, IGFBP5, β-glycoprotein-11, TIMP-3, EGR-2, p53, and others.

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Surprisingly, one of the most abundant proteins in the central nervous system, myelin proteolipid protein (PLP) (23), is up-regulated in leiomyomata (Table 1), even though myelin basic protein and 10 other myelin-related genes on the arrays did not differ between L and M.

The up-regulation in L of phosphatidylinositol 3-kinase and insulin receptor subtrate-1 (Table 1) is most interesting and seems to interconnect with pathways that have expanded the traditional (nuclear) mechanism of estrogen action (24, 25).

Genes Down-Regulated in Leiomyomata

Table 2 shows 52 of 78 genes that are down-regulated by more than two-fold in leiomyomata. Prominent among them are alcohol dehydrogenases 1α–γ, tryptase, dermatopontin, thrombospondin, coxsackievirus receptor, nur77, and c-kit (Table 2). Transcription factors such as c-fos, ATF3, orphan receptor TR3 or Nur77, and glucocorticoid receptors α and β were reduced in leiomyomata, as were several proteins involved in cell-cell contact and regulation of cytoskeletal rearrangement (ARHGEF6, GTPase guanylate-binding protein 2, cadherin-13, MAP kinase kinase kinase 5, tenascin-X, extracellular-matrix protein 2).

The coxsackievirus and adenovirus receptor (CAR) is also down-regulated in L. The decrease in L of CAR seems counterintuitive, if one would even consider a viral etiology for L. The natural ligand of CAR is unknown (26), and it was shown recently that CAR participates in epithelial cell-cell interactions and the formation of tight junctions (27, 28). CAR may have a role in myometrial contractions that are abolished in leiomyomata (see Discussion section).

Down-regulation in L of tryptase and mast cell carboxypeptidase A, both markers of mast cells (29), suggests that the number of mast cells is more greatly reduced in L than M. Depletion of mast cells has been observed in other tissues either by increased PPARγ2 expression (30) or a loss-of-function mutation in c-kit, which concomitantly depletes the c-kit$^+$ interstitial cells of Cajal (31).

Such data could be relevant to leiomyomata, which have increased PPARγ protein levels (9) and decreased c-kit mRNA levels relative to myometrium (Table 2); preliminary Western blots suggest that c-kit protein levels could also vary with the phase of the cycle (data not shown). The c-kit protein was detected in human endometrium (32) and myometrium (33), along with its ligand, the stem cell factor (34). In the arrays, stem cell factor is equally expressed in L and M.

Different forms of alcohol dehydrogenase 1 (α, β, γ), tryptase, and the glucocorticoid receptor are down-regulated in L, but only one accession number is provided in Table 2. The melanocyte specific gene 1 (msg1) was found to be both up- and down-regulated in leiomyoma; two accession numbers are given for different RNA transcripts, which, apparently, are expressed differently in leiomyoma than myometrium.

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DISCUSSION

Our longstanding interest in the development of leiomyomata prompted the study of surgical specimens using the microarray approach. Leiomyoma are well suited to analysis with this method since the growths arise from myometrium, a control tissue that is available from the same patient. The microarray approach has the attractive features of screening simultaneously a great number of genes in an unbiased fashion.

The fact that the same probes of a gene showed intense hybridization among the nine uterine pairs tested suggests that similar RNA phenotypes characterize leiomyomata from different patients, perhaps involving a common precursor or progenitor cell. This result is supported by cytogenetic studies that show identical cytogenetic abnormalities in different fibroids from the same patient (35). We interpret the pattern of overexpressed and underexpressed genes to suggest that leiomyomata may reflect an abnormal differentiation of uterine cells.

Undoubtedly, small differences (30%–60%) in mRNA levels between L and M could still translate into significant differences at the protein level or biological function, as convincingly shown with PPARγ and RXRα, where Northern blots gave L = M in the proliferative phase, whereas at the protein level, the ratio L:M was 4 (9).

Similarly, for the estrogen receptor α the arrays gave L:M = 1.5, which excludes it from Table 1, in agreement with classical hybridization experiments (36).

Variability among the nine patients in the ratios L:M (Tables 1 and 2, patient 5) could be attributed to polygenic traits and other factors, such as diet (phytoestrogens, retinol, etc.) that could not be evaluated in this study. Also, in the case of low mRNA expression in L or in M, subtracting the mismatch values from the perfect match for L and for M may be too stringent a correction. Western blots will clarify whether such large fluctuations also occur at the protein level.

Some gene products warrant further comment. Based on the increased levels of all-trans retinoic acid, PPARγ, and RXRα found in human leiomyomata (9), a number of the up-regulated genes (e.g., dlk, myelin PLP, CRABP II) would complement our proposed mechanism of leiomyoma growth.

Dlk is a transmembrane protein containing EGF-like repeat motifs homologous to the notch/delta/serrate family originally identified in Drosophila (37, 38). Dlk, also known as preadipocyte factor-1 (Pref-1), is an inhibitor of adipocyte differentiation (39, 40), whereas PPARγ is an inducer of adipocyte differentiation (41). It is intriguing that dlk participates in the callipyge phenotype of (skeletal) muscle hypertrophy in sheep (42).

A few genes listed in Table 1 are associated with polymorphisms (apolipoprotein E) or mutations (semaphorin F) found in neurological disorders or mental retardation. Some genes may also belong to a neural network associated with pain in leiomyomata, as was shown for pelvic adhesions (43).

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Up-regulation in leiomyomata of imprinted genes dlk, MEST, and IGF2 suggests that clusters of genes may link to leiomyoma growth (44, 45) and that differentiation of smooth muscle cells may be altered in leiomyomata. To date, there are approximately 40 maternally and paternally imprinted genes known (46). Loss of imprinting plays a role in oncogenesis, but no loss of IGF2 imprinting occurs in leiomyomata (45). Interestingly, dlk (Pref-1) and IGF2 were singled out in array studies of adenomyosis in newborn mice, which concluded that adenomyosis may be caused primarily by defects in myometrial differentiation and development (47).

Besides IGF2, IGF1, the IGF-receptor, and IGF binding proteins (IGFBPs) had been studied in human myometrium and leiomyomata (48–50) and in lympangioleiomyomatosis of the lung, which is more common in women than men (51).

PLP, which contains covalently bound fatty acids, was up-regulated in oligodendrocyte cultures by a PPARγ-PPARδ agonist (52) and might participate in leiomyoma growth regulation.

Frizzled, another transmembrane receptor found to be overexpressed in leiomyomata, has been shown to be involved in cardiac myocyte differentiation (21). Wnt proteins (ligands of the frizzled receptors) induced myocyte aggregation and adhesion with concomitant increases in N-cadherin. Direct cell-to-cell contact was required, and Wnt-induced aggregation could be abolished by anti-N-cadherin antibody (21). Our observation of increased frizzled and N-cadherin in leiomyomata may suggest that aggregation of myometrial cells may involve a similar signaling pathway, a possibility currently under investigation.

It has been postulated (53) that mast cells mediate myometrial contractions in the nonpregnant human uterus. Sivridis at al. (54) showed that mast cells concentrate in the junctional zone of the myometrium (just below the endometrium), which, as routinely seen by ultrasound, contracts reversibly during the cycle (55). Interestingly, anatomic studies have suggested that this region of the uterus may be particularly prone to leiomyoma development (56).

It is possible that c-kit–immunopositive cells (mast cells and the putative myometrial interstitial cells of Cajal) could assist or even function as the pacemaker cells of myometrial contractions, the latter being lost in leiomyomata. Based on the trans-differentiation of interstitial cells of Cajal to smooth muscle cells, as seen in newborn mice treated with c-kit neutralizing antibodies (31, 57), we speculate that in human myometrial smooth muscle cells, loss of c-kit expression might be relevant to leiomyoma initiation and growth.

A recent report on RNA editing in brain tumors (58) could shed an entirely new light on array results in general and, in particular, the puzzling finding that the ionotropic glutamate receptor subunit 2, or GluR-B, is up-regulated in leiomyomata relative to myometrium (Table 1). GluR-B is a glutamate-gated cation channel best studied in neuronal tissues that harbors the crucial RNA editing site, which confers low Ca^{2+}-permeability to the AMPA-type glutamate receptors (58).

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Post-transcriptional editing of pre-mRNA and tRNA was discovered a decade ago and is carried out by RNA-dependent deaminases; it enables the cell to recode genomic information by deleting, inserting, substituting, or modifying bases (59). Such base changes have a profound effect on the protein sequence translated from the edited mRNA. Maas et al. found that GluR-B mRNA, which is nearly 100% edited, was underedited in glioblastoma multiform relative to normal tissues and may correlate with tumor progression (58).

In conclusion, gene arrays have provided new data to expand the traditional approach to uterine fibroids. We interpret these data to suggest that many of the gene products up-regulated in leiomyomata are a manifestation of abnormally differentiated growth, whereas down-regulated genes mainly reflect loss in leiomyomata of myometrial contractions.

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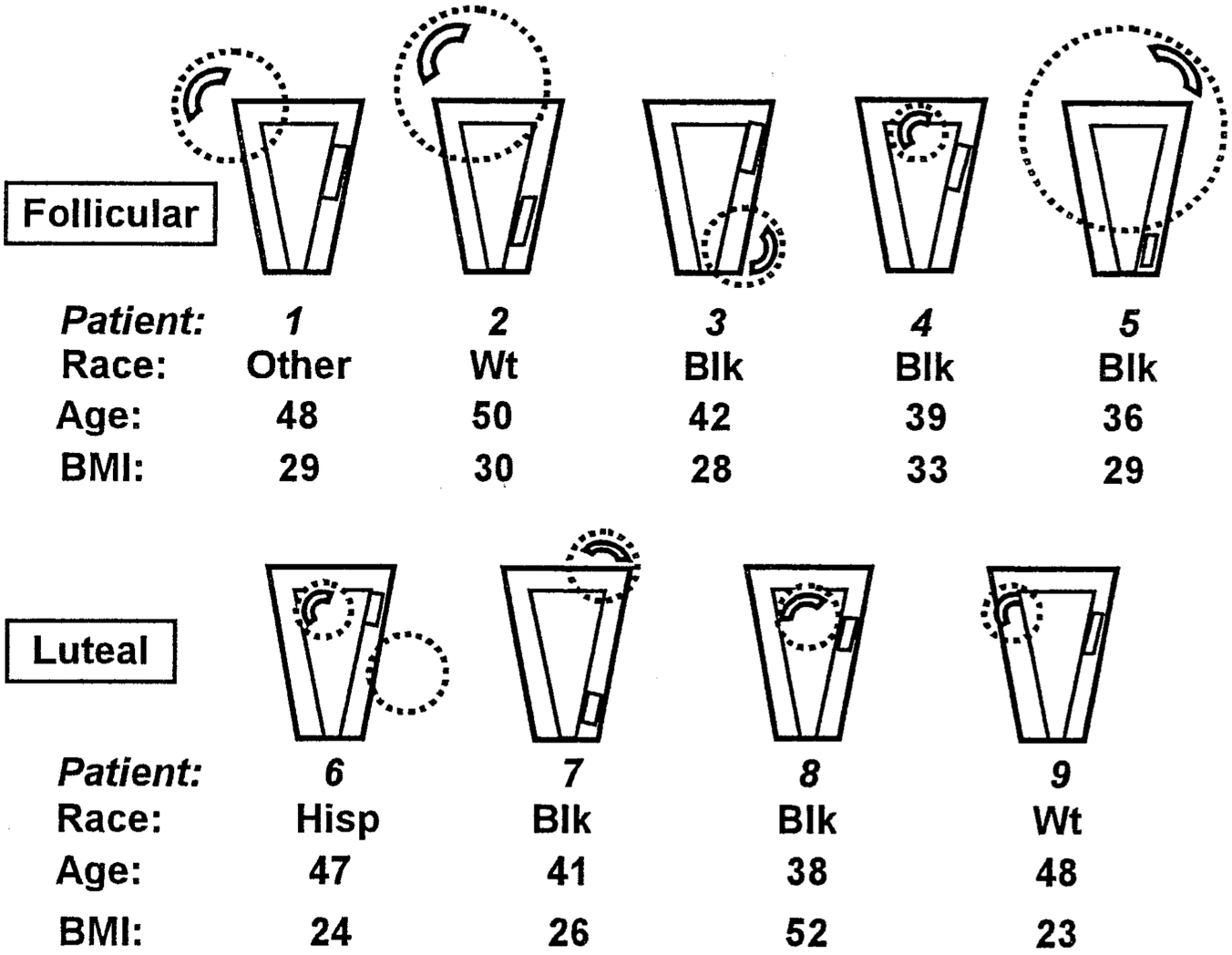

FIGURE 1.

Patient information and location of uterine tissues collected at the follicular or luteal phase of the menstrual cycle (Wt = white; Blk = black; Hisp = Hispanic). In this uterine cross-section diagram, leiomyoma and myometrial samples are shown by an arch and a rectangle, respectively.

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TABLE 1

Genes up-regulated by more than two-fold, on the average, in leiomyoma (L) relative to matched myometrium (M) from nine patients (L1:M1 to L9:M9).

Accession	L1:M1	L2:M2	L3:M3	L4:M4	L5:M5	L6:M6	L7:M7	L8:M8	L9:M9	Mean	Name
U15979	~47.5	~48.8	~3.9	~8.2	~434	~78	~2.5	1.3	5.9	70.01	Dlk (Delta-like 1) or Pref-1
AJ003112					~136	23	~15.2	~60.8	21.9	51.42	Doublecortin
AI093155					~148	34.7	4.7	3.2	12.9	40.74	JM27 or PAGE-1
L20814	3	−1.2	3.8	11.8	~195	50	58.1	~16.8	11.6	38.77	Ionotropic glutamate receptor, unit B
M12529	12.6	3.6	3	5.3	3.6	2.5	~51.6	~92.6	7.5	20.26	Apolipoprotein E3
J03242	3.3	6	5.9	9.7	52.1	19.6	25.9	3.2	25.3	16.78	IGF2
U52840	~14	~8	~19.2	~12.6	~11.5	~9.9	~30.4	~9.4	~27	15.78	Semaphorin F homolog
L27559	8.7	2.2	1.2	2.3	2.2	15.3	~37.2	~24.2	27.9	13.47	IGF binding protein 5 (IGFBP5)
M54927	17.9	2.1	19.1	30.8	17.3	4.7	6.2	2.3	8.4	12.09	Myelin proteolipid protein 1
D78611	16.1	7.5	3.1	2.5	15.4	~24.2	15.8	5.9	13.6	11.57	Mesoderm specific transcript MEST7PEG1
D00596	4.5	~35.7	14.5	7.7	~7.3	5.6	~2	~1.8	3	9.12	Thymidylate synthetase
AB011103					7.4	7.4	3.8	5.1	21.4	9.02	Kinesin KIF5B
L37882	9.9	26.4	13.8	12.6	2.8	2.7	3.6	3.4	2.1	8.59	Frizzled-2 (wnt receptor)
L33930	1.9	5.6	7.7	13.3	1.6	1.9	11.5	17.3	15.5	8.48	CD24 signal transducer
U52969	19.2	−2.1	9.2	2.3	6.3	17.2	6.2		7.5	8.23	Purkinje cell protein 4 (PCP4)
AL050404	~−3	~−1.8	~1.7	~2.9	8.7	~19.5	23.3	5.9	16.4	8.18	K^+ voltage-gated channel G1 (KCNG1)
U29195	~3.5	~4.5	~15.7	~7.8	~17.7	~4.2	~7.5	−1.5	~13.7	8.12	Neuronal pentraxin II (NPTX2)
U03106					21.1	4.1	~11.7	2.1	1.2	8.04	P21/WAF1 cyclin-kinase inhibitor
L11329	18.7	1.6	12.6	15.4	7.9	4.8	2	−1.9	7.2	7.59	Protein tyrosine phosphatase PAC-1
U79247	~8.1	~3.3	~5.5	~17.9	~10.6	~13.2	~4.1	−2.3	~2.4	6.98	Protocadherin PCDH11
×12949					3.1	~10.6	~10	2	2	5.54	Ret proto-oncogene
D82345	1.6	3	3.5	~19.3	1.3	3	8.9	4.2	4.8	5.51	Neuroblastoma thymosin β
J00073	15.5	−1.6	10.6	3	4.9	1.8	12.3	−2.8	3.5	5.24	α-cardiac actin
M97815	2	1.8	2.9	2.2	4.1	20.2	10.4	−1.4	3.9	5.12	Retinoic acid-binding protein II (CRABP-II)
X57766	3.9	1.3	7.4	10.7	1.1	5.9	8.7	1.1	5.5	5.07	Stromelysin-3
Z48481	2.3	3	2.6	2.1	−1.6	~7.2	~14.4	~2.5	~12.6	5.01	Matrix metalloproteinase 14
AF037335					2.4	6.1	10.6	−1.6	6.5	4.80	Carbonic anhydrase precursor (CA 12)
U32989	~2.8	~3.3	~3.8	~9	10.3	3.1	3.7	1.7	3.7	4.60	Tryptophan 2,3-dioxygenase

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Accession	L1:M1	L2:M2	L3:M3	L4:M4	L5:M5	L6:M6	L7:M7	L8:M8	L9:M9	Mean	Name
AL022718					8.5	2.9	2.4	1.1	7.2	4.42	DOC4-like protein
M65062	11.4	3	1.6	1.5	2.7	6.1	5.1	3.7	3.6	4.30	IGF binding protein 5 (IGFBP-5)
Y00067	~11.4	−1.1	4.7	1.7	1.7	~1.1	~6.7	5	3.5	3.86	Neurofilament subunit M
U16306	2.5	3.9	7.7	4.5	4.3	3	6.4	−1.6	3.4	3.79	Versican
U19599					4.2	3.3	4.7	3.2	3.4	3.76	BAX-delta (Bcl-2 gene family)
M11718	5.7	2.4	4.5	2.5	4.7	4.4	3.4	1.4	2.6	3.51	Collagen α2(V) (COL5A2)
U65092	2	~7.5	7.2	8.9	~2.3	~−1.4	~3.4	~−1.2	~2.1	3.42	Melanocyte-specific gene 1 (domain 1)
X14885	2.2	3.6	6.2	3.1	2.1	1.5	8.8	−3.1	6.3	3.41	TGFβ3
U89942	4.9	2.4	4.9	3.6	1.4	5.4	8.2	−3.5	2.9	3.36	Lysyl oxidase-like 2 (WS9-14)
U89281					1.2	2.2	8	1.9	3.4	3.34	Retinol dehydrogenase
Y14690					4.7	4.4	3.4	1.4	2.6	3.30	Collagen α2(V) (COL5A2)
AB029037					3.2	6.9	1.5	2.2	2.7	3.30	Trophinin
J05070	9	2.3	2.4	7.6	~−1.1	~2.1	~3.9	~−1.4	~4.8	3.29	Collagenase IV
HT2405	~3.1	~3.2	~6.3	4.5	2.9	1.5	3.6	1.2	3.2	3.28	IGF-lb
S42303	1.2	1.9	~9.9	4.7	3.8	1.9	1.6	1.2	1.6	3.09	N-cadherin
L12350	2	3.7	5.9	2.6	2.1	3.2	4.5	1.6	1.8	3.04	Thrombospondin 2
×54942	2.3	1.4	4.6	3.7	3.2	1.5	3.7	3.8	2.8	3.00	Cksl (cyclin-dependent kinase)
AF047469	~6.9	1.8	~7.2	~5.8	−1.9	1.6	1.5	1.8	1.8	2.94	Arsenite translocating ATPase
X70683	4	3.4	3.8	5.2	−1.1	−1.2	4.2	3.8	3.2	2.81	SOX-4 (HMG family SOX and Sry)
U30521	2.2	1.7	3.8	2.5	2.7	2.8	3.3	2.2	4.1	2.81	P311 (retinoic acid up-regulated gene)
U21551	2.8	5.4	2	6.6	1.2	1.3	1.7	2.1	1.9	2.78	ECA 39 (c-myc regulated)
S74445	~5.2	~2.5	~4.2	2.3	~7	~1.3	~1.7	~−2.3	~2.3	2.69	Cellular retinoic acid-binding protein
U30255	6.4	2.8	3.8	3.6	1.3	1.6	1.9	1.1	1.5	2.67	Phosphogluconate dehydrogenase
D78012	2	1.2	1.9	2	1.2	4.5	3.8	2.2	4.7	2.61	Dihydropyrimidinase related protein-1
M61906	1.2	3.7	1.6	2.3	1.2	2.6	3.5	1.3	2.5	2.21	Phosphatidylinositol 3-kinase p85
S62539	2	2.5	3.6	2.1	1.7	1.6	2.2	1.6	2.3	2.18	Insulin receptor substrate-1

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TABLE 2

Genes down-regulated by more than two-fold, on average, in L relative to M among nine patients.

Accession	L1:M1	L2:M2	L3:M3	L4:M4	L5:M5	L6:M6	L7:M7	L8:M8	L9:M9	Mean	Name
M12963	~−45.9	−23.5	~−62	~−68.2	−12.1	~−69.5	~−54.9	−3.6	−20.6	−40.03	Alcohol dehydrogenase ADHl-α, -β, γ
M30038					−2.9	−32.2	~−62.5	−7.1	−16.8	−24.30	Tryptase
Z22865	~−16	−6.1	~−8.5	~−10.2	−11.2	~−27.3	~−32.1	~−34	~−22.8	−18.69	Dermatopontin
X14787	−2.2	−5.3	2.4	1.9	~−72.6	−2.9	−3.4	~−41.2	−2.6	−13.99	Thrombospondin
M63438	~−3.4	−7.1	−4.8	~−70.6	3.4	1.6	−27.6	−1.9	−2.2	−12.51	Ig rearranged γ chain (V-J-C region)
AB002409	1.1	−8.9	−2.2	−1.9	−4.7	~−18.5	~−14.9	~−47.6	−7.2	−11.64	Chemokine CCL21 (or SLC)
M73720	−3.1	−1.4	−10.5	−10.2	−3.5	−12.2	~−15.8	−4.2	−12.7	−8.18	Mast cell carboxypeptidase A
V01512	3.2	−32.9	−6.6	−10.4	1.3	−8.9	−12.2	−4	−3	−8.17	c-fos
Y00503	−4.7	~−14.2	~−19.1	−3.4	−6.1	~−5.5	−5.5	~−4.5	~−9.8	−8.09	Keratin 19
K03000	−5.8	−8	−5	−5.6	−1.3	−17.9	−20.9	−3.2	−4.1	−7.98	Aldehyde dehydrogenase 1 (ALDH1)
U89337	−8.8	−18.9	−6	−3.9	−3.8	−2.2	−6.6	−3.4	−2.9	−6.28	Tenascin-XB [CREBL1, P450c21B]
L34058					−12.3	−2.5	−3.8	−9.4	−2.4	−6.08	Cadherin-13 (T- or H-cadherin)
L19871	−1.8	−10	−3	−3.2	−1.2	−11.8	−15.4	−1.5	−6.5	−6.04	Activating transcription factor 3 (ATF3)
AB020629					1.1	~−7.7	~−8.2	−4.1	~−9.6	−5.70	ATP-binding cassette subfamily A
D25304	~−13.4	1.4	~−16.5	~−12.9	−1.2	−2.2	−2.7	−1.1	−2	−5.62	GTPase Cdc42 or ARHGEF6
M55543	−1.5	1.8	−1.7	−1.4	−3.5	−6.9	−2.1	1	−35	−5.48	GTPase Guanylate-binding protein 2
×79510	−1.3	~−16.6	−5.3	−3.8	~−6.1	−1.3	−3.8	~−9.2	−1.5	−5.43	Tyrosine phosphatase D1
J02973	1.1	~−10.5	−4.6	~−7.5	−1.7	~−6	−2.6	~−12.7	−3.6	−5.34	Thrombomodulin
X07523					3.9	−8.4	−7.8	−12.5	−1.9	−5.34	Complement factor H
Y11307	−1.5	−10.4	−6.4	−6.5	−1.3	−3.6	−7.1	−7.5	−3.4	−5.30	Cyr61, connective tissue factor
M62402	−3.4	−1.1	−2	−7.2	1.2	−14.5	−16.3	−1.7	−2.3	−5.26	IGF binding protein 6 (IGFBP-6)
X15357	−1.6	−2.7	−2.8	−1.6	−2.9	~−4.6	−3.8	−7.7	~−15.5	−4.80	α-Atrial natriuretic peptide receptor
Y07593					−7.1	−1.7	~−8.5	−1.5	−5	−4.76	Coxsackievirus receptor
U03877	~−4.8	~−5	~−3.4	~−7.2	−2.9	−6.8	−6.5	−1.5	−2.5	−4.51	Extracellular matrix fibulin-3
×83857	−3.8	−1.4	−17.7	−7.1	−1.2	−2.5	−3.1	−1.3	−2.2	−4.48	Prostaglandin E2 receptor EP3al
L22524	−4.7	−5.5	−4	1	−9.7	−10.1	−1.1	−4.4	−1.1	−4.40	Matrilysin (matrix metalloproteinase)
M25079	−1.5	−4.7	−1.6	−2.8	−2.1	−12.3	−9.6	−1.1	−3.7	−4.38	Sickle cell β-globin
X05908	−4.3	−2.4	−3.7	−6.7	−1.6	−6.2	−6.6	−4.4	−3	−4.32	Annexin Al

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Accession	L1:M1	L2:M2	L3:M3	L4:M4	L5:M5	L6:M6	L7:M7	L8:M8	L9:M9	Mean	Name
AF030186					−1.7	~−4.5	~−6.1	~−5.7	−3.5	−4.30	Glypican-4
D31883	−2	−1.6	−3.7	−1.9	−2.7	−3.6	~−15.7	−1.7	~−5.6	−4.28	Actin binding LIM protein 1
M89796	−3	−2.5	~−5.6	~−7.7	~−4.4	~−3.7	~−3	~−3.4	−5	−4.26	IgE receptor, β chain
D86096	−1.3	−1.5	−7.1	−5.6	−1.4	−5.9	−10.3	−2.1	−2.6	−4.20	Prostaglandin E receptor EP3-IV
M74587	−2.2	−1.5	−1.1	−2.1	~−24.5	−1.6	−1	−1.6	−1.7	−4.14	IGF binding protein 1 (IGFBP-1)
U67156	−2.8	−3.7	−3.1	−1.5	−7	−6.1	−5.1	−1.2	−6.7	−4.13	MAP kinase kinase kinase 5
J03764	−1.7	−1.5	1.2	−1.6	~−5.2	−6.9	−5.2	−14.3	−1.6	−4.09	Plasminogen activator inhibitor 1
J03507	−13.6	−4.5	−2.9	−1.2	3.7	−1.8	−4.3	−4.9	−5.2	−3.86	Complement component 7
M63967	1.2	−4.7	−1.7	−1.5	~−10.6	−5	−1.5	−9.4	−1.2	−3.82	Aldehyde dehydrogenase ALDH1B1
HG1428HT1428	−1.6	−2.9	−3.3	−3.5	−1.4	−9.8	−7.5	−1.3	−2.9	−3.80	Globin, beta
L13740	−1.3	−4.8	−1.2	−1.9	−1	−7.3	−5.5	−7.3	−3.7	−3.78	Nur77 (TR3 receptor)
L06139	~−3.8	−1.9	~−8.5	~−7.7	−1.2	−2.6	−4.2	−1.4	−2.3	−3.73	TEK tyrosine kinase
ABO 11792					−1.8	−5.7	−6.7	−1.7	−1.8	−3.54	Extracellular matrix protein 2
M98539	−1.7	−9.2	−3.5	−6.7	−4.1	−4.9	−2	2.1	−1.2	−3.47	Prostaglandin D2 synthase
J04152	−3.4	~−2.5	~−3.6	~−3.4	−8.2	−1.7	−2.7	−2.6	−2.8	−3.43	Tumor antigen GA733-1
J02611	1.1	−3.1	−1.5	−2.3	−2.4	−7.9	−7.9	−1.8	−4.8	−3.40	Apolipoprotein D
Z19585	−2.1	−5.6	−1.7	−1.7	−5.2	−8.9	−2.9	−1.2	−1.2	−3.39	Thrombospondin 4
AL021977					−2.6	−2.9	−5	−2	−4.4	−3.38	Maff protein (v-maf)
D37931	−1.6	−1.2	−1.3	−2.5	−3.8	−12.8	−3.2	−2.2	−1.8	−3.38	RNase A-4
U65093	−1.9	−3.3	−3.4	−1.8	−1.9	−2.9	−5	−3.2	−2.1	−2.83	MSG1-related gene 1 (domain 2)
X06182	−4.3	−2.2	~−12	−1.6	~−3.4	~−2.1	~1.2	~1.5	1.2	−2.41	c-kit (receptor of stem cell factor)
U85267	−1.1	−1.9	−2.5	2.1	1.4	−5.2	−2.8	−7.8	−3.7	−2.39	Downs syndrome gene DSCR-1
HG4582-HT4987	−2.3	−2.7	−3.5	−2.8	1.1	−2.6	−6	−1	−1.7	−2.39	Glucocorticoid receptors β/α
D50683	−2	2.5	−2	−3.2	−1.2	−2.4	−5.5	−1.7	−2.7	−2.02	TGFβ receptor II