Estrogen-Associated Genes in Uterine Leiomyoma

SHUANFANG LI AND JOHN A. McLACHLAN

Department of Pharmacology, Tulane University, and Center for Bioenvironmental Research at Tulane & Xavier Universities, SL-3, 1430 Tulane Avenue, New Orleans, Louisiana 70112, USA

ABSTRACT: Uterine leiomyomas or fibroids are classified as a benign uterine disease in that the polyps display no malignant growth. However, uterine leiomyomas are a leading cause of morbidity, infertility, and hysterectomy in women. Since leiomyomas are known to be sensitive to estrogen for their growth, we have examined uterine genes known to be estrogen responsive in affected and unaffected uterine tissue. This information will be useful in determining the contribution, if any, of hormonally active environmental chemicals to this highly prevalent reproductive disorder.

KEYWORDS: estrogen; estrogen receptor; leiomyoma; pregnancy; tumor; uterine leiomyoma

INTRODUCTION

Uterine leiomyomas are very common benign tumors in women of reproductive age. It is estimated that more than 40% of women above the age of 35 have a leiomyoma. Enlargement of leiomyoma causes serious gynecological problems, such as pelvic pain, menorrhagia, dysmenorrhea, reduced fertility, and recurrent pregnancy loss.[1,2] This disease is not only a leading cause of morbidity in women, but is also a major justification for hysterectomies, which account for more than 200,000 cases a year.[3,4]

As a tumor from the estrogen target organ (uterus), uterine leiomyoma is considered an estrogen-associated tumor. Evidence supporting this includes its development after menorrhea, enlargement and growth during pregnancy, and regression after menopause. Most directly, estrogen receptor alpha (ER-α) is more highly expressed in the leiomyoma than in the myometrium.[5,6] Decrease of ovarian estrogen secretion by gonadotropin release hormone (GnRH) agonist reduces tumor volume.[7,8]

Address for correspondence: Dr. John A. McLachlan, Center for Bioenvironmental Research at Tulane/Xavier Universities, SL-3, 1430 Tulane Avenue, New Orleans, LA 70112-2699.Voice: 504-585-6910; fax: 504-585-6428.
john.mclachlan@tulane.edu

TABLE 1. Summary of estrogen-associated genes in uterine leiomyoma

Gene	Expression	Phase difference	Estrogen regulation
Receptors			
ER-α	Up	Follicular	Yes
ER-β	Down	No	Yes
ER-α/β	Up	Follicular	Yes
PR(B)	Up	Uncertain	Yes
IGF-I	Up	Uncertain	Yes
PPAR(γ)	Up	NA	Uncertain
Aromatase growth factors	Up	Uncertain	No
EGF	Up	Luteal	Yes
IGF-1	Up	Follicular	Yes
IGF-II	Up	Follicular	Uncertain
PTH-R	Up	Follicular	Yes
Cell cycle related			
C-jun, C-fos	Down	No	Yes
Ki-67	Up	Follicular	Yes
PCNA	Up	Follicular	Yes
Channels			
Connexin 43	Up	Uncertain	Yes
Others			
Endothelin-1A	Up	Uncertain	Yes
Nitric oxide	Up	Uncertain	Uncertain
HMG (C,Y)	Up	Uncertain	Uncertain
Wnt 7a	Down	No	Uncertain

SOURCE: Anderson,[34] Gokdeniz *et al.*,[52] Gutavsson *et al.*,[53] Sumitani *et al.*,[54] Walker *et al.*,[55] Wang *et al.*,[56] Li *et al.*,[57] and Chiang *et al.*[58]

Many estrogen genes have been studied in the leiomyomas. TABLE 1 summarizes those genes and their expression in leiomyoma, expression change during menstrual cycle, and their association with estrogen. In this paper we present our recent research results on estrogen receptor β (ER-β), peroxisome proliferator-activated receptors (PPAR), and Wnt 7a, as well as methylation-associated genes in leiomyoma.

ESTROGEN RECEPTORS α AND β IN UTERINE LEIOMYOMA

Since the cloning of the ER in 1986,[9,10] it has been believed that only a single receptor (now termed ER-α) was responsible for mediating the effects of estrogen on target tissues. Estrogens such as estradiol 17β bind with ER-α, and again this binding complex binds to the estrogen response element (ERE) in the 5′ region of the open reading frame of the DNA to modulate the transcription of the target gene. Re-

cently, a new subtype of ER, ER-β, was identified.[11–13] Although ER-β has DNA sequences that are very similar to ER-α and shares some functions with ER-α, in many cases it plays a very different role from ER-α. Studies have shown that in addition to ER-α, ER-β might play an important role in the pathophysiology of the disease of the steroid hormone target organs. The change of relative expression level of ER-β to ER-α might contribute to the development of breast and ovarian cancer.[14–16] We have compared the relative expression of ER-α and ER-β in leiomyomas and the adjacent myometria. By comparing the semiquantitative results of ER-α/β mRNA and immunohistochemistry generated from RT-PCR amplification, we found that the expression of ER-α was higher than that of ER-β in both leiomyoma and normal myometrium; ER-α expression was increased in leiomyoma compared to that in the adjacent normal myometrium; ER-β expression was the same or even lower in leiomyoma than in the adjacent normal myometrium. In the 36 cases studied, 61% showed an increased ratio of ER-α to ER-β in leiomyoma. These results suggest that in addition to ER-α, ER-β may play an important role in the development of leiomyoma and that an imbalance in expression of these receptors may contribute to the pathogenesis of the disease. Experiments are being conducted to see the effects of changing the expression level of ER-β in the leiomyomal cell lines.

OVEREXPRESSION OF PEROXISOMAL PROLIFERATER-ACTIVATED RECEPTORS IN LEIOMYOMA

The peroxisome proliferator-activated receptors (PPARs) alpha, beta/delta, and gamma are novel nuclear hormone receptors activated by long-chain fatty acids and synthetic ligands and which regulate lipid metabolism.[17,18] PPAR forms a binding complex with peroxisome proliferators (PPs), which are a diverse class of chemicals, including the lipid- and cholesterol-lowering fibrate drugs (clofibrate, fenofibrate, and gemfibrozil), leukotriene antagonists, herbcides, plasticizers, solvent, and naturally occurring chemicals (e.g., phenyl acetate) or hormones.[19,20] PPARs form heterodimers with the 9-*cis* retinoic acid receptors, RXRα. These heterodimers bind to peroxisome proliferator response element (PPRE) located in the 5′-flanking region of target genes. The toxic and carcinogenic action of PP have been shown in rodents. Recent studies showed that peroxisome proliferation in estrogen target tissues might be triggered by estrogens, leading to carcinogenesis. Considering that the peroxisome proliferation can be triggered in estrogen target tissues by estrogens and PPs, the role of PPARs in leiomyoma should be clarified. We have demonstrated an increased expression of PPARα in leiomyoma as compared to its adjacent myometrium (TABLE 2), which is not the case in PPARβ and γ. Recently, Tsibris *et al.*[21] reported a variant PPARγ mRNA level in the menstrual cycle that was lower in leiomyoma than myometrium during the luteal phase and higher in leiomyoma than myometrium during the follicular phase. They hypothesized that in the follicular phase, E2, unopposed by progesterone, is the primary stimulus for increased PPARγ expression.[21]

While the reason is uncertain, obesity has been associated with the high rate of leiomyoma among black women.[22,23] The increased risk of uterine leiomyoma is thought to be due to the higher level of circulating estrogens in obese women. Epi-

TABLE 2. IHC detection of PPARs in uterine leiomyoma and the adjacent myometrium

Number	PPARα			PPARβ			PPARγ		
Staining	++	+	−	++	+	−	++	+	−
Leiomyoma									
Without Gn-RH									
38	32	4	2	6	20	12	5	10	23
With Gn-RH									
8	2	6	0	0	4	2	0	1	7
Adjacent myometrium									
40	4	34	2	0	10	30	0	9	31

++: strong; +: positive; −: negative.

demiological evidence suggests that a high-fat diet promotes the development of obesity and that there is a direct relationship between the amount of dietary fat and the degree of obesity. Obesity has an important influence on the development of a variety of morbidities among African American women. It shows that approximately 50% of adult African American women are considered obese by prevailing standards. Moreover, this number appears to be increasing.[24,25] The reasons for this very high prevalence of obesity among African American women are unknown. Fibroids are two to three times more common, develop earlier, and grow larger in African American women than in white women. Diet habit, such as higher dietary fat intake, may be one of the factors influencing obesity and therefore, development of the leiomyoma.

Interestingly, peroxisome proliferation in estrogen target tissues can be triggered by estrogens.[26] Recent studies also showed that the vitellognin A2 estrogen response element (ERE) can function as a PPAR response element (PPRE) and is bound by a PPAR/RXR (retinoid X receptors) heterodimer,[27,28] suggesting the signaling cross-talk between PPAR/RXR and estrogen receptor. Therefore, PPAR activation caused by estrogen may be one of the ways in which the gene regulates and affects the formation and progress of the uterine leiomyoma.

On the basis of our results, we proposed a hypothesis of leiomyoma development via PPAR pathway. PPAR activation can be induced by (1) exposure to peroxisome proliferators (PPs), (2) absorption of dietary lipid, and (3) increased estrogen levels. PPAR activation can then induce a variety of adaptive changes. Two of these changes, peroxisome proliferation-induced oxidative injury and enhanced cell replication, may potentially contribute to the development of leiomyoma. Some environmental factors have been noted in the etiology of uterine leiomyomas.[29–32] Most of these factors are environmental estrogens, such as DES, DDT/DDE, bisphenol A, dioxins, PCBs, and alpha hexachlorocyclohexane (HCH). However, no studies on the association of PPs and leiomyoma have been found. The role of PPs in the development of the leiomyoma needs to be investigated in the future.

DECREASED EXPRESSION OF WNT7A MRNA LEVEL AND ITS INVERSE ASSOCIATION WITH ESTROGEN RECEPTOR-α IN LEIOMYOMA

Pathologically, uterine leiomyomas are composed of whorled, anastomosing fascicles of uniform, fusiform smooth muscles. The cells are spindle-shaped and have abundant fibrillar, eosinphilic cytoplasm as well as distinct borders. Nuclei are elongated with blunt or tapered ends, and have finely dispersed chromatin with small nuclei. The tumor generally remains benign and rarely transforms into a malignant type—leiomyosarcoma.[33] Biologically, leiomyoma cells express myometrium-specific genes such as actins, myosins, and desmins. A high level of steroid receptors, altered fibroblast growth factor, and transforming growth factor-beta has been found in leiomyoma cells.[6,34] Clonality studies have shown that multiple tumors in the same uterus develop a *de novo* mechanism.[35] Considering the pathological appearance and biological characteristics of the leiomyoma, we postulate that the leiomyomas are a mass of uterine myometria that loses control of pattern/formality. This idea prompted us to study the patterning genes in the development of leiomyoma. The candidate gene we have studied was Wnt 7a. Wnt genes are a large family of highly conserved, developmentally related genes.[36] The vertebrate Wnt genes are homologous to *wingless*—the *Dorosophila* segment polarity gene that encodes a secreted molecule implicated in the patterning and establishment of cell boundaries during embryogenesis. The term "Wnt" is derived from the combination of *wingless* and *int-1*, which was found to be a common integration site of mouse mammary tumor virus (MMTV) in mammary epithelial carcinomas.[37,38] Wnt genes are not only involved in cell–cell communication and patterning, but also associated with oncogenesis, such as sex steroid hormone-responsive cancers, including breast and uterine carcinoma.[39–41] Among the 16 family members identified in the vertebrate, the Wnt7a gene not only guides the development of the anteroposterior axis in the female reproductive tract, but also plays a critical role in uterine smooth muscle patterning and maintenance of adult uterine function.[42–44] In mice, loss of Wnt7a expression results in several notable abnormalities in the female reproductive tract. The uterus lacks glands and the epithelium is stratified compared to the normal cytoarchitecture of wide-type female. In addition, an overgrown, poorly differentiated myometrium has been observed in Wnt7a knock-out mice.[45] These features resemble pathological appearances in leiomyoma. Furthermore, Wnt7a is also responsive to changes in the levels of sex steroid in the female reproductive tract.[46–48] Such evidence has persuaded us to explore the role of the Wnt7a gene in the pathogenesis of human leiomyoma.

With semiquantitative PCR, of 30 leiomyomas studied, 67% showed a decreased mRNA level of Wnt7a as compared to the paired myometrium. However, ER-α mRNA was hyperexpressed in 67% of the leiomyomas as compared to their paired myometrium. An inverse association at mRNA expression was found between Wnt7a and ER-α.[49]

The inverse association of the Wnt7a and ER-α mRNA suggests a deregulation mechanism by which the steroid hormones regulate the expression of Wnt7a in leiomyoma. Although the means by which estrogens mediate the expression of Wnt7a is unclear, studies have observed a suppression of Wnt7a by estrogens. Miller *et al.*

MFL1
GGGGCGCGAGCGATCCGAAACAACGAGGCGCGGTGGAGAAGAACCCATGATGTCAGGCAGGGCTTACCTTTGCGGAT
AACCCGGATATCGATGGGCTGAATGATGGTGATTAGACGAACGCGGATAAATGCCTATAATCAGACATCACTATTAA
TTGCAGCCGAACAATCAGAATTTACGGTTGTGCTACAAAGTGTCCATGCTCAAGGCCGATCTTGTTGCACTGCGAAA
ACCCGTAGTCGCAGCTTTCCTGATCGCGCCCC (263bp)

MFL2
GGGGCGCGACGGCACGGGTCACGACTGGCCGTGGTGGCAGCAGATGGCCGCCAAGTTCCTGTAAATTCGCAAGCCTG
GCGCTTCGCCTCACTGGATCGCTTGCGCGGTCGCGAGGATGCTGAGATCCTTGACTCAAGGCGGGTCAGCGCCAGGA
GGATCGATGAAGAGGATCGGGATTGTCTACGGGATGGGGAACTCCTTCCCGGGGGCATTGGTGGACAAGATTAATTC
GATGGGCATCGCCGGCGTTGCGGCGGGGCACGTCAAGATTGGCGCCATCCGGATGGACGAAAAATGTCCATACCGGG
TGATCATCGACCGGATTTCTCACGATATTTCCGTTCTATCGCGCCCG
(355bp)

FIGURE 1. Methylation fragments in leiomyoma. *Underline*: primer region.

have shown that fetal exposure of diethylstilbestrol (DES), a synthetic estrogen, can result in deregulation of Wnt7a during murine uterine morphogenesis.[45] Referring to their results, we have postulated that hypersensitivity of leiomyoma cells to estrogen may deregulate the Wnt7a expression. Decreased expression of Wnt7a may lead to loss of control in patterning of the myometrium and result in development of leiomyoma.

METHYLATION-ASSOCIATED GENES IN LEIOMYOMA

Chromosomal rearrangements, translocations, and deletions have been observed in uterine leiomyomas.[10–12] However, a correlation exists between leiomyoma size and the presence of cytogenetic abnormalities.[50,51] This suggests that rearrangements occur in already existing tumors and are secondary events in leiomyoma tumor progression. The initial event in the pathogenesis of the leiomyoma is not yet understood. DNA methylation change, without change of the DNA sequence, plays an important role in gene transcription regulation. Aberrant methylation has been found in the process of many diseases and cancer. In order to understand the role of methylation in the development of the leiomyoma, we have used a recently developed PCR-based method,[21] called methylation-sensitive restriction fingerprint (MSRF), to screen abnormally methylated CpG sites in human leiomyoma. We have identified and cloned two genomic fragments that frequently undergo methylation changes in leiomyoma. Fragment 1 is 269 bp and fragment 2 is 355 bp (FIG. 1). A BLAST search has revealed no significant matches between the known sequences in the database and the two sequences. The two fragments are hence designated as novel hypermethylation-containing fragments in human uterine leiomyoma and are named methylation fragments in leiomyoma (MFL-1 and MFL-2) (gene-bank access No. AZ081761; AZ081762). Further studies of the hypermethylated genes in the development of the leiomyoma will be illuminating.

REFERENCES

1. CRAMER, S.F. & A. PATEL. 1990. The frequency of uterine leiomyomas. Am. J. Clin. Pathol. **94:** 435–438.

2. CRAMER, S.F., J.A. HORISZNY & P. LEPPERT. 1995. Epidemiology of uterine leiomyomas: with an etiologic hypothesis. J. Reprod. Med. **40:** 595–600.
3. WILCOX, L.S., L.M. KOONIN, R. POKRAS, *et al.* 1994. Hysterectomy in the United States, 1988–1990. Obstet. Gynecol. **83:** 549–555.
4. LEPINE, L.A., S.D. HILLIS, P.A. MARCHBANKS, *et al.* 1997. Hysterectomy surveillance—United States, 1980–1993. Morb. Mortal. Wkly. Rep. CDC Surveillance Summary **46:** 1–15.
5. OTSUKA, H., M. SHINOHARA, M. KASHIMURA, *et al.* 1989. A comparative study of the estrogen receptor ratio in myometrium and uterine leiomyomas. Int. J. Gynaecol. Obstet. **29:** 189–194.
6. BENASSAYAG, C., M.J. LEROY, V. RIGOURD, *et al.* 1999. Estrogen receptors (ERalpha/ERbeta) in normal and pathological growth of the human myometrium: pregnancy and leiomyoma. Am. J. Physiol. **276:** E1112–E1118.
7. BROEKMANS, F.J. 1996. GnRH agonists and uterine leiomyomas. Hum. Reprod. **11**(Suppl 3): 3–25.
8. BARBIERI, R.L. 1999. Ambulatory management of uterine leiomyomata. Clin. Obstet. Gynecol. **42:** 196–205.
9. GREEN, S. & P. CHAMBON. 1986. A superfamily of potentially oncogenic hormone receptors [news]. Nature **324:** 615–617.
10. GREENE, G.L., P. GILNA, M. WATERFIELD, *et al.* 1986. Sequence and expression of human estrogen receptor complementary DNA. Science **231:** 1150–1154.
11. KUIPER, G.G., E. ENMARK, M. PELTO-HUIKKO, *et al.* 1996. Cloning of a novel receptor expressed in rat prostate and ovary. Proc. Natl. Acad. Sci. USA **93:** 5925–5930.
12. HYDER, S.M., C. CHIAPPETTA & G.M. STANCEL. 1999. Interaction of human estrogen receptors alpha and beta with the same naturally occurring estrogen response elements. Biochem. Pharmacol. **57:** 597–601; also Erratum Biochem. Pharmacol. **57**(12):1447, 1999.
13. COWLEY, S.M., S. HOARE, S. MOSSELMAN & M.G. PARKER. 1997. Estrogen receptors alpha and beta form heterodimers on DNA. J. Biol. Chem. **272:** 19858–19862.
14. LEYGUE, E., H. DOTZLAW, P.H. WATSON & L.C. MURPHY. 1998. Altered estrogen receptor alpha and beta messenger RNA expression during human breast tumorigenesis. Cancer Res. **58:** 3197–3201.
15. SPEIRS, V., A.T. PARKES, M.J. KERIN, *et al.* 1999. Coexpression of estrogen receptor alpha and beta: poor prognostic factors in human breast cancer? Cancer Res. **59:** 525–528.
16. PUJOL, P., J.M. REY, P. NIRDE, *et al.* 1998. Differential expression of estrogen receptor-alpha and -beta messenger RNAs as a potential marker of ovarian carcinogenesis. Cancer Res. **58:** 5367–5373.
17. CATTLEY, R.C., J. DELUCA, C. ELCOMBE, *et al.* 1998. Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? Regul. Toxicol. Pharmacol. **27:** 47–60.
18. CATTLEY, R.C. & R.A. ROBERTS. 2000. Peroxisome proliferators and carcinogenesis: editorial perspectives [editorial]. Mutat. Res. **448:** 117–119.
19. WILLSON, T.M., P.J. BROWN, D.D. STERNBACH & B.R. HENKE. 2000. The PPARs: from orphan receptors to drug discovery. J. Med. Chem. **43:** 527–550.
20. SLADEK, R. & V. GIGUERE. 2000. Orphan nuclear receptors: an emerging family of metabolic regulators. Adv. Pharmacol. **47:** 23–87.
21. TSIBRIS, J.C., K.B. PORTER, A. JAZAYERI, *et al.* 1999. Human uterine leiomyomata express higher levels of peroxisome proliferator-activated receptor gamma, retinoid X receptor alpha, and all-trans retinoic acid than myometrium. Cancer Res. **59:** 5737–5744.
22. SATO, F., M. NISHI, R. KUDO & H. MIYAKE. 1998. Body fat distribution and uterine leiomyomas. J. Epidemiol. **8:** 176–180.
23. SHIKORA, S.A., J.M. NILOFF, B.R. BISTRIAN, *et al.* 1991. Relationship between obesity and uterine leiomyomata. Nutrition **7:** 251–255.
24. KJERULFF, K.H., P. LANGENBERG, J.D. SEIDMAN, *et al.* 1996. Uterine leiomyomas. Racial differences in severity, symptoms and age at diagnosis. J. Reprod. Med. **41:** 483–490.

25. FAERSTEIN, E.S.M. & N. ROSENSHEIN. 2001. Risk factors for uterine leiomyoma: a practice-based case-control study. Am. J. Epidemiol. **153:** 1–19.
26. MA, H., H.W. SPRECHER & P.E. KOLATTUKUDY. 1998. Estrogen-induced production of a peroxisome proliferator-activated receptor (PPAR) ligand in a PPAR gamma-expressing tissue. J. Biol. Chem. **273:** 30131–30138.
27. KELLER, H., F. GIVEL, M. PERROUD & W. WAHLI. 1995. Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements. Mol. Endocrinol. **9:** 794–804.
28. NUNEZ, S.B., J.A. MEDIN, O. BRAISSANT, *et al.* 1997. Retinoid X receptor and peroxisome proliferator-activated receptor activate an estrogen responsive gene independent of the estrogen receptor. Mol. Cell. Endocrinol. **127:** 27–40.
29. HOUSTON, K.D., D.S. HUNTER, L.C. HODGES & C.L. WALKER. 2001. Uterine leiomyomas: mechanisms of tumorigenesis. Toxicol. Pathol. **29:** 100–104.
30. HUNTER, D.S., L.C. HODGES, P.K. EAGON, *et al.* 2000. Influence of exogenous estrogen receptor ligands on uterine leiomyoma: evidence from an in vitro/in vivo animal model for uterine fibroids. Environ. Health Perspect. **108**(Suppl. 5): 829–834.
31. VOLLENHOVEN, B. 1998. Introduction: the epidemiology of uterine leiomyomas. Baillieres Clin. Obstet. Gynaecol. **12:** 169–176.
32. SAXENA, S.P., C. KHARE, A. FAROOQ, *et al.* 1987. DDT and its metabolites in leiomyomatous and normal human uterine tissue. Arch. Toxicol. **59:** 453–455.
33. ZALOUDEK, C.J.N.H. 1994. Mesenchymal Tumors of the Uterus: 488–494. Springer-Verlag. New York.
34. ANDERSEN, J. 1998. Factors in fibroid growth. Baillieres Clin. Obstet. Gynaecol. **12:** 225–243.
35. TOWNSEND, D.E., R.S. SPARKES, M.C. BALUDA & G. MCCLELLAND. 1970. Unicellular histogenesis of uterine leiomyomas as determined by electrophoresis by glucose-6-phosphate dehydrogenase. Am. J. Obstet. Gynecol. **107:** 1168–1173.
36. NUSSE, R. & H.E. VARMUS. 1992. Wnt genes. Cell **69:** 1073–1087.
37. NUSSE, R., A. VAN OOYEN, D. COX, *et al.* 1984. Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. Nature **307:** 131–136.
38. NUSSE, R., A. BROWN, J. PAPKOFF, *et al.* 1991. A new nomenclature for int-1 and related genes: the Wnt gene family [Lett.]. Cell **64:** 231.
39. FUKUCHI, T., M. SAKAMOTO, H. TSUDA, *et al.* 1998. Beta-catenin mutation in carcinoma of the uterine endometrium. Cancer Res. **58:** 3526–3528.
40. HUGUET, E.L., J.A. MCMAHON, A.P. MCMAHON, *et al.* 1994. Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue. Cancer Res. **54:** 2615–2621.
41. TEKMAL, R.R. & N. KESHAVA. 1997. Role of MMTV integration locus cellular genes in breast cancer. Front. Biosci. **2:** d519–d526.
42. MILLER, C., K. DEGENHARDT & D.A. SASSOON. 1998. Fetal exposure to DES results in de-regulation of Wnt7a during uterine morphogenesis [letter]. Nat. Genet. **20:** 228–230.
43. PARR, B.A. & A.P. MCMAHON. 1998. Sexually dimorphic development of the mammalian reproductive tract requires Wnt-7a. Nature **395:** 707–710.
44. SASSOON, D. 1999. Wnt genes and endocrine disruption of the female reproductive tract: a genetic approach. Mol. Cell. Endocrinol. **158:** 1–5.
45. MILLER, C. & D.A. SASSOON. 1998. Wnt-7a maintains appropriate uterine patterning during the development of the mouse female reproductive tract. Development **125:** 3201–3211.
46. BUI, T.D., M. LAKO, S. LEJEUNE, *et al.* 1997. Isolation of a full-length human WNT7A gene implicated in limb development and cell transformation, and mapping to chromosome 3p25. Gene **189:** 25–29.
47. BUI, T.D., L. ZHANG, M.C. REES, *et al.* 1997. Expression and hormone regulation of Wnt2, 3, 4, 5a, 7a, 7b and 10b in normal human endometrium and endometrial carcinoma. Br. J. Cancer **75:** 1131–1136.
48. MILLER, C., A. PAVLOV & D.A. SASSOON. 1998. Differential expression patterns of Wnt genes in the murine female reproductive tract during development and the estrous cycle. Mech. Dev. **76:** 91–99.

49. LI, S., T.C. CHIANG, G.R. DAVIS, *et al.* 2001. Decreased expression of Wnt7a mRNA is inversely associated with the expression of estrogen receptor-alpha in human uterine leiomyoma. J. Clin. Endocrinol. Metab. **86:** 454–457.
50. NIBERT, M. & S. HEIM. 1990. Uterine leiomyoma cytogenetics. Genes Chromosomes & Cancer **2:** 3–13.
51. REIN, M.S., W.L. POWELL, F.C. WALTERS, *et al.* 1998. Cytogenetic abnormalities in uterine myomas are associated with myoma size. Mol. Hum. Reprod. **4:** 83–86.
52. GOKDENIZ, R., S. OZEN, B. MIZRAK & N. BAZOGLU. 2000. GnRH agonist decreases endothelial nitric oxide synthase (eNOS) expression in leiomyoma. Int. J. Gynaecol. Obstet. **70:** 347–352.
53. GUSTAVSSON, I., K. ENGLUND, M. FAXEN, *et al.* 2000. Tissue differences but limited sex steroid responsiveness of c-fos and c-jun in human fibroids and myometrium. Mol. Hum. Reprod. **6:** 55–59.
54. SUMITANI, H., M. SHOZU, T. SEGAWA, *et al.* 2000. In situ estrogen synthesized by aromatase P450 in uterine leiomyoma cells promotes cell growth probably via an autocrine/intracrine mechanism. Endocrinology **141:** 3852–3861.
55. WALKER, C.L., K.D. BURROUGHS, B. DAVIS, *et al.* 2000. Preclinical evidence for therapeutic efficacy of selective estrogen receptor modulators for uterine leiomyoma. J. Soc. Gynecol. Invest. **7:** 249–256.
56. WANG, H.S., T.H. WANG & Y.K. SOONG. 2000. Elevation of insulin-like growth factor-binding protein-1 mRNA expression following hormone replacement therapy. Hum. Reprod. **15:** 50–54.
57. LI, S.D.G., R. WILLIAMS & J. MCLACHLAN. 1999. Overexpression of peroximal proliferater-activated receptor alpha (PPARa) in Human Uterine Leiomyoma. Presented at the 81st meeting of the Endocrine Society, San Diego, CA.
58. CHIANG. T. R.-D.G., C. WALKER, J. MCLACHLAN & S. LI. 2000. Comparative expression of estrogen receptor a and b in uterine leiomyoma and the adjacent normal myometrium. Presented at the AACR Annual Meeting, San Francisco, CA.