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Quantitative determination of sst2 and sst5 gene expression in uterine leiomyomata and the effect of treatment with somatostatin analogue

Uterine leiomyomata are the most common tumors in women over 30 years of age. There is increasing evidence that growth factors may play a role in the generation and/or growth of this tumor. Growth hormone (GH), insulin-like growth factor-I and -II (IGF-I and IGF-II) were recently implicated; GH receptors were found in leiomyomata and myometrium (1), and a greater abundance of IGF-I and IGF-II mRNA and IGF-I receptors were found in fibroids than in normal myometrium (2). Interestingly, it has been recently shown that acromegaly, a disorder characterized by high levels of GH, is associated with a high incidence of uterine leiomyomata (3).

We recently demonstrated that administration of the somatostatin analogue lanreotide leads to a reduction in uterine and myoma volume in fertile women. The mean reduction was about 24% for total uterus dimension and 41.6% for myoma volume (4).

In the present report, we determined the quantitative expression of somatostatin receptor 2 and 5 (sst2 and sst5) genes in leiomyomata and normal myometrium, and a case of a woman with leiomyomata treated with somatostatin analogue is reported.

Leiomyoma tissue and homologous myometrium were isolated from women with leiomyomata who underwent myomectomy or hysterectomy at our departments. The operations were in general performed because of clinical symptoms related to leiomyoma disease. All patients were healthy without any gynecological disease that would interfere with the results. Tissue samples were collected from seven women. Small strips of myometrium from the edge of the incisions were collected from two women who were undergoing elective cesarean delivery at the 38th week of pregnancy. Three women were fertile and two were postmenopausal. None of the postmenopausal women had received any hormonal medication for at least 3 months before surgery.

Three tissue samples were run per patient. All samples were snap-frozen in liquid nitrogen and stored at −70°C. The remaining specimen was fixed in buffered formaldehyde solution, and routine histological diagnosis was performed. All samples were run in triplicate. The primers and probe for sst2 mRNA quantification to use with the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) were selected by the proprietary software Primer Express on the sequence NM_001050 (GeneBank).

The forward primer corresponds to the region from base 448 to base 466 (sequence 5′-TCGGCCAAGT-GGAGGAGAC-3′). The reverse primer corresponds to the region from base 491 to base 510 (sequence 5′-AGAGACTCCCCACACAGCCA-3′). The internal oligonucleotide probe is labeled with FAM at the 5′ end and with TAMRA at the 3′ end and has the sequence 5′-(FAM)-CCGGACGGCCAAGATGATCACC-(TAMRA)-3′.

The primers and probe for sst5 mRNA quantification to use with the ABI Prism 7700 Sequence Detection System were selected by the proprietary software Primer Express on the sequence NM_001053 (GeneBank).

The forward primer corresponds to the region from base 734 to base 751 (sequence 5′-TCCTCTC-CTACGCCAACAGC-3′). The reverse primer corresponds to the region from base 789 to base 811 (sequence 5′-GGAAGCTCTGGCGGAAGTT -3′). The internal oligonucleotide probe is labeled with FAM at the 5′ end and with TAMRA at the 3′ end and has the sequence 5′-(FAM)-CCCGTCCTCTACGGCTTCCTCTCTGA-(TAMRA)-3′. Four thousand nanograms of total RNA were reverse-transcribed by random examiners following the classical reverse transcription Perkin-Elmer protocol (5).

A 43-year-old woman with leiomyomata was treated with lanreotide for 3 months. Lanreotide depot (30 mg) was administered every 14 days for 3 months. Nuclear magnetic resonance (NMR) was performed before and after therapy. The patient did not use sex steroids or GnRH analogs less than 1 year before the start of the study. The woman was informed of the aim of the treatment and its experimental nature and gave her written consent. Uterine and myoma volume was calculated by applying the equation for the volume of an ellipsoid ($L \times W \times D \times \pi/6$). The study was approved by the Institutional Review Board of the University of Siena.

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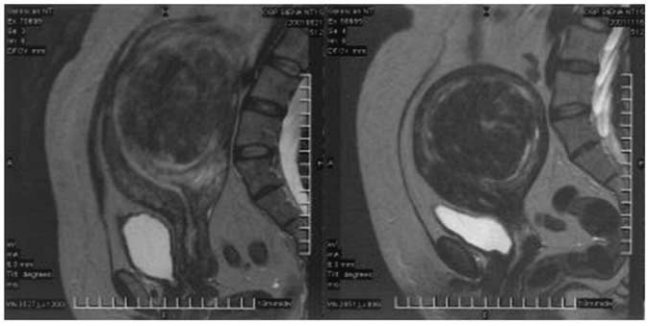
FIGURE 1

Total uterine volume before (left) and after (right) lanreotide treatment.



De Leo. Lanreotide therapy and uterine leiomyomata. Fertil Steril 2003.

A wide range of sst2 and sst5 gene expression was found in normal myometrium and leiomyomata (range: 1.04–8.8 and 1.1–9.3 $\times$ 10^7 mRNA/μg total RNA, respectively). The sst2 and sst5 mRNA expression was similar in leiomyoma and surrounding myometrium. A trend for decreased expression in myometrium from postmenopausal women and increased expression during pregnancy was evident. No significant correlation was found between sst2 and sst5 gene expression.

A woman with uterine leiomyomata was treated with the somatostatin analogue, lanreotide. Normal E_2 and FSH plasma levels indicated the fertile status of the patient. Lanreotide treatment was not followed by any modification in E_2 and FSH. Total uterine volume was 3,297 cm^3 before and 1,766 cm^3 after therapy (percentage of reduction: 46%; Fig. 1). The three myomas measured 575, 67, and 33 cm^3 before therapy and 217, 12, and 8 cm^3, after therapy, respectively (percentage of reduction, 62%, 82%, and 75%, respectively). The patient reported an improvement in abdominal pressure and in the quantity of menstrual bleeding.

Increasing evidence suggests that not only ovarian hormones but also GH and growth factors may be important in the maintenance of leiomyomata. It was recently demonstrated that women with acromegaly, a disorder characterized by high plasma concentrations of GH, have an 81% prevalence of leiomyoma (3).

Shrinkage of leiomyoma after lanreotide treatment may be explained by the relevant suppression of GH-IGF axis after somatostatin analogue administration. Alternatively, somatostatin should act on myometrium and leiomyoma by binding specific receptors. Indeed, we have identified receptors sst2 and sst5 in both normal myometrium and myoma. This finding could explain the efficacy of somatostatin analogue treatment.

The antiproliferative effect of somatostatin is determined in part indirectly through inhibition of the release of mitogenic hormones and growth factors, through the inhibition of angiogenesis, and in part directly through ssts located on cell membranes (6). Among the different sst subtypes recently identified (sst1–sst5), sst2 mediates the antiproliferative effect more efficiently than the others (7, 8). Somatostatin binds with high affinity to all sst subtypes, whereas the currently commercially available octapeptide analogues bind with a high affinity only to sst2 and sst5 (9).

The present study provides important evidence that the GH-IGF system plays a pathogenic role in maintaining uterine fibromyomas and that somatostatin analogue may be an effective new therapy for this condition.

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