Transforming growth factor β1 expression in uterine leiomyomas and adjacent myometrium

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Objective: To analyze the expression of transforming growth factor (TGF)β1 in human uterine leiomyomas and adjacent myometrium obtained at hysterectomy of women at mid/late follicular phase (n = 15), at mid/late luteal phase (n = 12) and after sex steroid ablation therapy. **Materials and methods:** Sex steroid ablation therapy was carried out using luteinizing-hormone-releasing hormone analog (triptorelin 3.75 mg intramuscularly every 28 days for at least 3 months; n = 10) and postmenopause (n = 8). The expression of TGFβ1 mRNA was analyzed by northern blots and *in situ* hybridization analysis while the immunohistochemical analysis assessed TGFβ1 expression at protein levels. In addition, we tested the time- and dose-dependent effects of TGFβ1 (0.5–50 ng/ml; final concentration; 24 and 48 h) on the proliferation (cell number; trypan blue exclusion) of KW smooth muscle-like myometrial cells *in vitro*. **Results:** Our data demonstrated that TGFβ1 (mRNA and protein) expression was higher in leiomyomas at mid/late follicular phase compared with those at luteal phase and to adjacent myometrium at either phases of the menstrual cycle. However, TGFβ1 expression (mRNA and protein) did not differ significantly among leiomyomas at luteal phase, leiomyomas after luteinizing-hormone-releasing hormone analog therapy, myometrium at follicular phase, myometrium at luteal phase and myometrium postmenopause. **Conclusion:** We conclude that TGFβ1 may be an important autocrine/paracrine factor in human leiomyomas particularly during the mid/late follicular phase.

Keywords: leiomyomas, lutenizing-hormone-releasing hormone analog, transforming growth factor β1

Uterine leiomyoma is the most common neoplasia in women. It is estimated that 20 to 30% of all women will eventually develop a uterine leiomyoma. This may have a negative impact on women's fertility as it represents the most common cause of hysterectomy of women during the reproductive years [1]. Uterine leiomyomas are rarely diagnosed before puberty as they increase in size during pregnancy and decrease in size after the menopause. Consequently, it has been suggested early on that sex steroid hormones may participate in the pathophysiology of the disease [2,3]. In addition, numerous studies have strongly supported the notion that uterine-derived growth factors can act as local mediators of sex steroid hormone action on uterine tissues as well as on other sex steroid target tissues [3–6]. As a result, a long list of growth factors have been investigated for possible causative implications in the pathophysiology of leiomyomas over the last 30 years [4–8]. Indeed, the transforming growth factor (TGF)β1–3 family and type I–III TGFβ receptor(.R)s have been the focus of intense investigation [9,10]. The exogenous administration of TGFβ1 has demonstrated that it exerts pivotal actions on myometrium smooth muscle cells *in vitro*, acting at different concentrations either as inhibitors or stimulators, depending on the presence or absence of other local growth factors [11]. However, studies focusing on TGFβ1 expression have presented contradictory data, suggesting that TGFβ1 mRNA expression is either similar between leiomyomas and adjacent myometrium at all phases of the menstrual cycle [12] or higher (20%) in human adjacent myometrium when compared with leiomyomas [11]. Interestingly, the latter study reported that the TGFβ1 bioactivity is remarkably increased (by 40%) in human myometrium at early/mid luteal phase (LP), possibly as a result of the regulatory or hormonal influences at this particular phase on pH and serine protease activity [11]. However, other investigators have documented that leiomyomas overproduce TGFβ1 and TGFβ.R when compared with myometrium [13–17]. In addition, sex steroid ablation therapy using luteinizing-hormone-releasing hormone analog (LHRH-A) was shown to downregulate TGFβ1 expression in human leiomyomas [13,15,17].

Herein we have investigated the effects of exogenous administration of TGFβ1 (1–25 ng/ml) on KW smooth muscle cell-like myometrial cells *in vitro* and analyzed TGFβ1 expression in human

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leiomyomas and adjacent myometrium of premenopausal women. Uterine tissues were selected at mid/late follicular phase (FP) and mid/late LP, as established by menstrual history and confirmed by histopathologic evaluation of the endometrium after hysterectomy. In addition, TGFβ1 expression was assessed in leiomyomas of women receiving LHRH-A (triptorelin; for at least 3 months) and of women postmenopause (for at least 3 years of the menopause).

Material & methods

Cell culture

KW smooth muscle-like myometrial cells have been previously characterized in our laboratory [18,19]. This cell line was grown in 75 cm^2 culture flasks using Dulbecco's modified Eagle's medium (DMEM)F/12 (Gibco BRL) containing 5% fetal bovine serum (FBS). KW cells were plated at a cell density of 2.5×10^4 cells in 24-well plates and grown with DMEM/F-12 containing 5% FBS. Cells were then exposed to TGFβ1 in a dose-dependent (1–25 ng/ml) and time-dependent manner (24 and 48 h). The number of KW cells was counted on a hematocytometer and their viability was determined by trypan blue exclusion assay [19].

Tissue biopsies

The uterine biopsies were obtained during hysterectomy of women who had previously signed an informed consent pre-approved by the local Ethics Committee. The phase of the reproductive cycle was calculated by the women's menstrual history (mid/late FP: 10–14 days; mid/late LP: 24–28 days) and confirmed by pathology evaluation of the endometrium after hysterectomy. Tissue biopsies of leiomyomas and adjacent myometrium selected to be in:

- Mid/late FP (n = 15; increasing estrogen levels/absent progesterone)
- Mid/late LP (n = 12; increased estrogen/progesterone levels
- greater than 3 months after sex steroid ablation therapy ($L_{LHRH\text{-}A}$; minimal levels of sex steroids), using a LHRH-A; triptorelin 3.75 mg, every 28 days
- Postmenopause (Mn; minimal levels of sex steroids). Tissues were collected at surgery and kept frozen (-80°C) until their use

TGFβ1 expression

Northern blots and *in situ* hybridization analysis were employed to assess TGFβ1 mRNA expression while immunohistochemistry was used to assess TGFβ1 expression at the protein level.

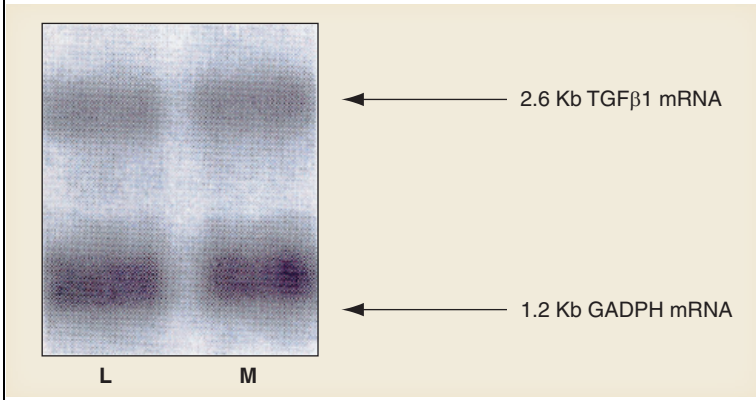
Northern analysis

Uterine tissues were homogenized and extracted with phenol/chloroform. The RNA was precipitated in 80% ethanol [20–22], and its concentration was determined spectrophotometrically at 260 and 280 nm. 10 µg poly-A mRNA was isolated in chromatography columns of oligo-dT cellulose, subjected to electrophoresis in 1.2% agarose gel and transferred onto Hybond N™ (Amersham) membrane. These membranes were pre-exposed and exposed in hybridization conditions prior to transfer. Blots were exposed to ^{32}P-labeled human TGFβ1 and Glyseraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA. $TGF\beta_1$ cDNA probe detected a 2.6 kB $TGF\beta_1$ mRNA in human tissues. Human GAPDH/TGFβ1 cDNAs were obtained by American Type Culture Collection (ATCC). Autoradiogramms were visualized on KODAK X-Omat RP films with Cronax lightning screens. The shadow density on films was analyzed with LKB 2222–020 Ultrascan XL laser densitometer. The basic expression of GAPDH (obtained from ATCC) was used as an internal marker. This probe traces a zone at 1.2 kB representing the expected size of GAPDH mRNA. Thus, the expression of TGFβ1 mRNA versus GAPDH mRNA was calculated for each sample (signal density) and the ratio of TGFβ1 mRNA:GAPDH mRNA was used for our calculations and the statistical analysis of the results [21,22].

In situ *hybridization*

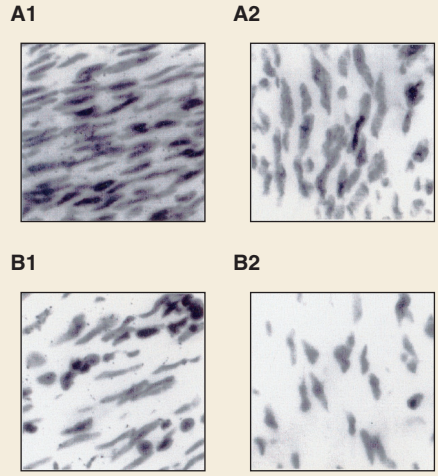
The leiomyomas and myometrial tissues were initially kept at -80°C and were then embedded in Optimal Cutting Temperature (OCT) compound and mounted onto a cryostat. Serial sections were cut (8 mm thick) at -20°C, collected on poly-L-lysine coated slides, fixed for 20 min in 4% paraformaldeyde (w/v) in 0.1 M phosphate buffer at 4°C and then washed in 0.1 M phosphate buffer for 20 min (4 times × 5 min each). Prior to hybridization, the sections were washed in 2 × SSC (0.3 sodium chloride and 0.03 sodium citrate) and then in a 2 × SSC solution containing 0.1% Triton X-100 for 10 and 10 min respectively. Prehybridization was performed in a buffer solution containing 50% (v/v) formamide 5 × SSPE (20 × SSPE = 0.18 M NaCl and 10 mm NaH_2PO_4), 1 mM ethylenediaminetetraacetic acid (EDTA) (pH

Figure 1. Northern blot detection of TGFβ1 mRNA and GADPH mRNA in human uterine tissues.



An example of the northern blots which detected the transforming growth factor (TGF)β1 mRNA (2.6 Kb) and GAPDH mRNA (1.2 Kb) in human uterine tissues. Radiograms were produced by the use of radioactive GAPDH and TGFβ1 cDNA probes (ATCC) and exposed to Kodak X-omat RP film with Cronax lightning screens for 5 days. Optical density was analyzed by a LKB 2222-020 Ultrascan XL laser densitometer. GAPDH expression was used as an internal marker for the analysis of relative TGFβ1 expression in uterine tissues.
ATCC: American Type Culture Collection; GADPH: Glyseraldehyde-3-phosphate dehydrogenase; L: Leiomyomas; M: Myometrium.

Figure 2. *In situ* hybridization signals, which reflected the expression of the TGFβ1 mRNA in uterine tissues.



A1: Expression of TGFβ1 mRNA in leiomyomas; **B1:** Expression of TGFβ1 mRNA in adjacent myometrium; **A2 & B2:** Represent the negative controls, respectively (RNAse A and RNAse T-treated tissue-sections, as described in Material and Methods section). The analysis of signal densities was by PhosphorImager, using the ImageQuant program.

7.4), 0.1% SDS, 0.1% (w/v) polyvinylpyrrolidone, 200 µg/ml denatured salmon testis DNA, 2 mg/ml poly(AMP), 4% (w/v) dextran sulfate and 10 mM DTT at RT for 2 h [20,21]. Concentration of 2 million counts of ^{35}S-labeled human TGFβ_1 probe was used. The radiolabeled TGFβ_1 cDNA probe was diluted in the prehybridization buffer and applied to each section. Hybridization was performed at 39°C for 16 h. After hybridization, the slides were washed at 20°C in

- 2 × SSC solution for 90 min at RT
- 1 × SSC solution for 90 min at RT
- 0.5 × SSC solution for 60 min at RT, 0.5 × SSC for 60 min in 37°C and 0.5× SSC for 60 min at RT

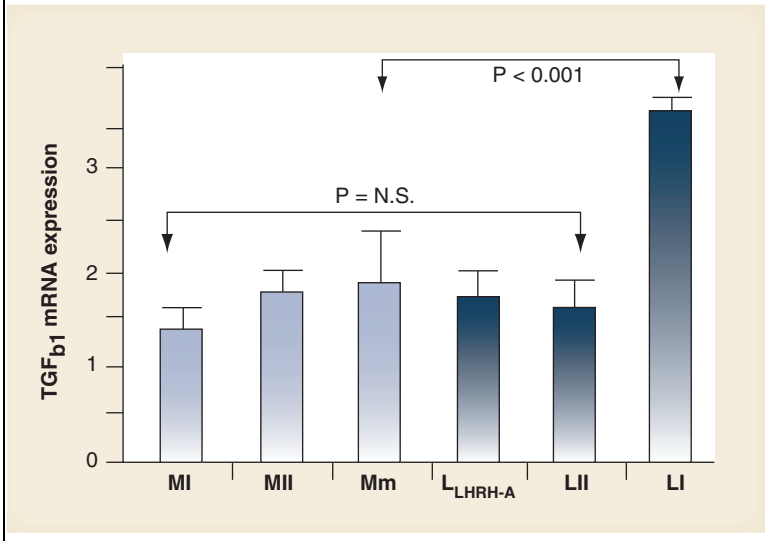
The slides were rinsed with ascending concentrations of ethanol (70, 90 and 100%), air dried and exposed to Kodak film for 14 days. The sections were coated with Kodak NTB-2 liquid photographic emulsion diluted 1:1 with distilled water at 45°C and stored in darkness at 4°C. After 28 days of exposure, the negative controls were developed and stained with H and E. The slides used as negative controls (RNAse) were pretreated with RNAse A (10 µg/ml) and RNAse T (100.000 units/ml) in 2 × SSC for 45 min at 37°C. Leiomyomal, myometrial and RNAsed slides (negative controls) were exposed to the

Table 1. The effects of the TGFβ1 on the proliferation of KW smooth muscle-like cells.

Number of living KW cells as assessed by trypan blue exclusion (% above control).		
ng/ml	***24 h***	***48 h***
0.5	-4	+1.0
1.0	+2	-2.0
10.0	-5	-15.5§
25.0	-18§	-25.0§
50.0	-23§	-32.5§

§$p < 0.05$; control cell count = 55.325 ± 956 cells (X ± SE).
TGF: Transforming growth factor.

Figure 3. Comparative analysis of the TGFβ1 mRNA expression as detected by the *in situ* hybridization in leiomyomas and adjacent myometrium of women in mid/late FP and mid/late LP of the menstrual cycle.



In addition, we present the analysis of TGFβ1 mRNA expression in leiomyomas after luteinizing-hormone-releasing hormone analog (L_{LHRH-A}) and myometrium post-menopause (Mm). TGFβ1 mRNA expression was significantly higher in leiomyomas (L) at mid/latefollicular phase (FP) than every other tissue tested. Statistical analysis was performed by the paired t-test and unpaired t-test, when appropriate, while nonparametric analysis, such as Wilcoxon's rank sum test, Mann–Whitney test and Kolmogorov–Smirnov test was used to confirm the data of the unpaired t-test.
LI: Mid/late follicular phase; LII: Mid/late luteal phase; L_{LHRH-A}:Luteinizing-hormone-releasing hormone analog; LP: Luteal phase; MI: Mid/late follicular phase; MII: Mid/late luteal phase.

same X-ray film. The intensity of the signals was analyzed using v3.0 (Molecular Dynamics). Signal intensity (absorbance/mm^2) was evaluated by subtracting the respective RNAse signal for each individual signal measurement in leiomyomas and myometrium tissues. The results were expressed as a ratio of the leiomyomas:myometrium signal intensity in tissues from each hysterectomy, based on the mean data of three different experiments on each tissue [19,20,23].

Immunohistochemistry

Uterine tissues were fixed using 10% buffered formalin for 48 h and fixed in paraffin blocks. Consecutive sections were cut with microtome using slides coated with poly-L-lysine. The slides, after deparafinization and rehydration in descending concentrations of ethanol, were washed in 95°C for 5 min in a solution of 10 mM of sodium citrate, pH 6.0, then in distilled water (three times for 2 min each). They were then incubated for 5 min and washed in PBS (twice for 5 min each). For the immunohistochemistry we used a TGFβ1 bovine polyclonal antibody (Santa Crouz) at a dilution of 1/25 using as a tracing system the ImmunoCrouz™ Staining System (Santa Crouz) with 3.3/diaminobenzidine (DAB) as a chromogen [19,20,24].

Statistical analysis

We used paired and unpaired t-test and nonparametrical methods (such as Wilcoxon's rank sum test, Mann–Whitney test and Kolmogorov–Smirnov test) for additional verification of the t-test results ($p < 0.05$).

Results

The effects of TGFβ1 on the cell count

Exogenous administration of increasing concentrations of TGFβ1 showed that it exerted a dose-dependent (0.5 up to 50 ng/ml) and time-dependent (24 and 48 h) inhibitory effect on the proliferation of KW cells in cultures containing 5% fetal bovine serum (**Table 1**).

TGFβ1 mRNA expression in leiomyomas & adjacent myometrium

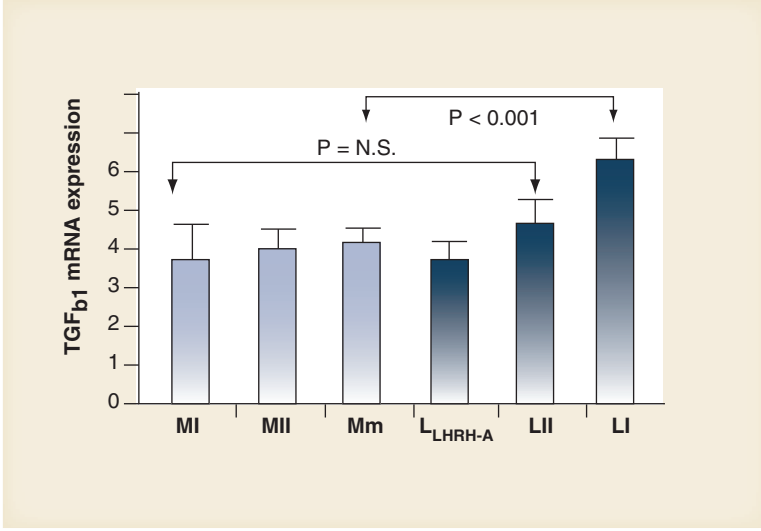
Northern analysis revealed that TGFβ1 and GAPDH cDNA probes depicted the expected 2.6 and 1.2 Kb bands, which correspond to TGFβ1 and GAPDH mRNAs, respectively (**Figure 1**).

In situ hybridization analysis revealed that radiography signals which correspond to TGFβ1 mRNA were detected around smooth muscle cells and vascular endothelial cells in leiomyomas and adjacent myometrium, documenting that smooth muscle cells did in fact express TGFβ1 (**Figure 2**).

Comparative analysis showed that *in situ* hybridization signals corresponding to the TGFβ1 mRNA expression were significantly more intense in leiomyomas at mid/late FP (LI) than that of leiomyomas at mid/late LP (LII), of adjacent myometrium at mid/late FP (MI) and myometrium at mid/late LP (MII) (LI > LII; LI > MI; and LI > MII [$p < 0.001$]) (**Figure 3**). However, comparative analysis of TGFβ1 expression showed that the intensity of *in situ* hybridization signals did not differ significantly among leiomyomas at LP (LII) and adjacent myometrium at either phases of the menstrual cycle (MI & MII) (LII vs MI; LII vs MII; MI vs MII [$p > 0.05$]) (**Figure 3**).

In addition, TGFβ1 mRNA expression of leiomyomas at FP (LI) was higher than that of leiomyomas after LHRH-A therapy (L_{LHRH-A}) and myometrium postmenopause (Mm) (LI > LLHRH-A and LI > Mm; [$p < 0.001$]). However, leiomyomas at LP (LII), leiomyomas

Figure 4. Comparative analysis of TGFβ1 mRNA expression as detected by northern blots in leiomyomas and adjacent myometrium of women in mid/late FP and mid/late LP of the menstrual cycle.



In addition, we present the analysis of TGFβ1 mRNA expression in leiomyomas after luteinizing-hormone releasing-hormone analog and myometrium postmenopause (Mm). TGFβ1 mRNA expression was significantly higher in leiomyomas (L) at mid/late folliculr phase (FP) than every other tissue tested. Statistical analysis was performed by the paired t-test and unpaired t-test, when appropriate, while nonparametric analysis, such as Wilcoxon's rank sum test, Mann–Whitney test and Kolmogorov–Smirnov test was used to confirm the analysis of the unpaired t-test.
LI: Mid/late follicular phase; LII: Mid/late luteal phase; L_{LHRH-A}: Luteinizing-hormone-releasing hormone analog; LP: Luteal phase; MI: Mid/late follicular phase; MII:Mid/late luteal phase.

after LHRH-A therapy, myometrium postmenopause and adjacent myometrium at either phases of the menstrual cycle contained similar TGFβ1 mRNA expression (LII vs L_{LHRH-A} vs Mn vs MI vs MII; [$p > 0.05$]) (**Figure 3**).

Northern blots confirmed *in situ* hybridization analysis, regarding the pattern of TGFβ1 mRNA expression in leiomyomas and adjacent myometrium during the menstrual cycle, after LHRH-A treatment and post-menopause (LI > LII [$p < 0.05$]; LI > MI; [$p < 0.001$]; LI > L_{LHRH-A} [$p < 0.001$]) and (LII vs L_{LHRH-A} vs MII vs MI vs Mn; $p > 0.05$) (**Figure 4**).

Immunocytochemical detection of the TGFβ1 expression

Semiquantitative analysis documented that TGFβ1 expression was increased in leiomyomas at mid/late FP as compared with leiomyomas at LP and myometrium at either FP and LP as well as to leiomyomas after LHRH-A. Similarly, TGFβ1 expression did not differ among biopsies of myometrium at any phase of the menstrual cycle and postmenopause (**Figures 5 & 6**).

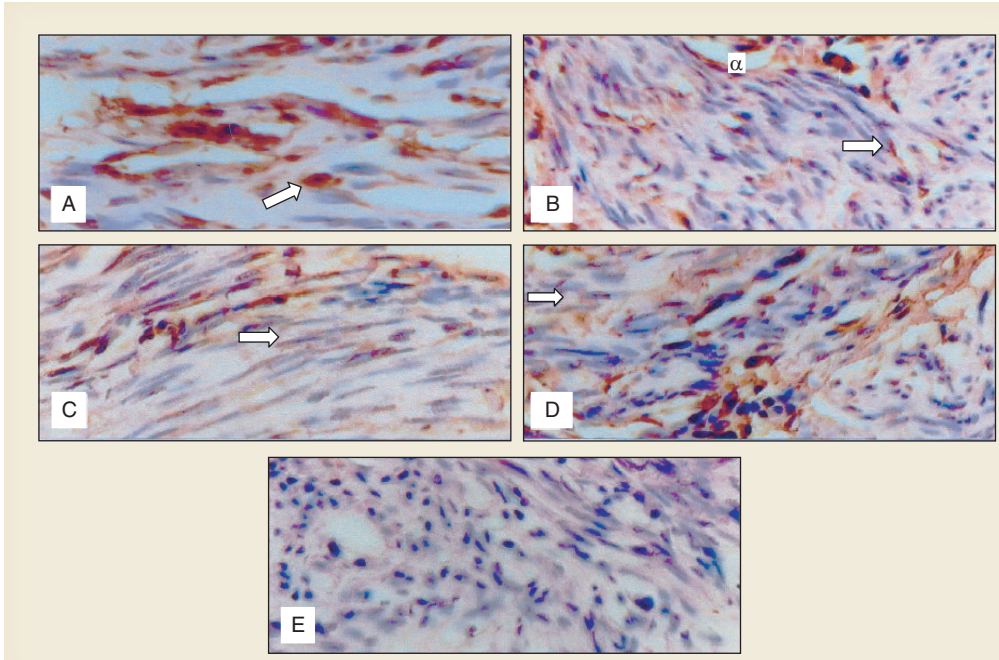
Discussion

The identity of molecular mechanisms that account for the myometrium cellular transformations, leading to the pathogenesis of leiomyomas are currently unknown. However, it is clear that ovarian steroids are essential for the evolution of leiomyomas thereafter [4]. Differential expression of bioactive molecules during the menstrual cycle, such as growth factors, have been considered to play a key role in leiomyomas growth, mediating sex steroid hormone actions on smooth muscle cells [4–7].

Recently, microarray analysis has identified several differentially expressed genes, including those of TGFβ1, TGFβ.R and their intracellular signaling pathways (Smads), which can mediate, at least in part, the altered cell biology of leiomyomas [25]. In addition, several studies have detected that TGFβ1 mRNA expression in uterine leiomyomas is increased as compared to adjacent myometrium and that sex steroid hormone ablation therapy, using LHRH-A, decreases TGFβ1 and TGFβ.R expression [13,15,17].

Since uterine tissues are composed of various cell types and fibrous components (especially leiomyomas/fibromas) and contain blood vessels known to express (vascular endothelial cells) TGFβ1, we have used *in situ* hybridization and immunohistochemical analysis to confirm that TGFβ1 is expressed at transcription and protein level by the smooth muscle cells of myometrium and leiomyomas. In addition, we used Northern analysis to confirm the *in situ* hybridization data in uterine tissues. Our data documented that leiomyomas contain higher TGFβ1 expression during the mid/late FL as compared with all myometrium and leiomyomas tissues tested in this study. These data have confirmed previous reports, which have reported higher TGFβ1 expression in uterine leiomyomas as compared with adjacent myometrium [13,15,17]. However, our data suggested that leiomyomas contained comparable TGFβ1 expression with the adjacent myometrium at mid/late LP. In addition, our data suggested that the TGFβ1 expression of adjacent myometrium is not significantly influenced by the profile of sex steroid hormones at mid/late FP (increasing estrogen levels/absent progesterone), at mid/late LP (increased estrogen/progesterone levels), and postmenopause (minimal sex steroid hormone activity). Moreover, TGFβ1 expression of leiomyomas was unaffected by the sex steroid hormone profile,

Figure 5. Immunochemical detection of TGFß1 expression in the smooth muscle cells and endothelial cells of the blood vessels in human uterine tissues.



A: TGFβ1 expression in leiomyomas at mid/late follicular phase (FP); **B:** TGFβ1 expression in adjacent myometrium at mid/late FP; **C:** TGFβ1 expression in leiomyomas at mid/late luteal phase (LP); **D:** TGFβ1 expression in myometrium at mid/late LP; **E:** Negative control (without anti-TGFβ1 antibody). Note that an increased TGFβ1 expression was noted in leiomyomas at mid/late FP of the menstrual cycle as compared with the other tissues.

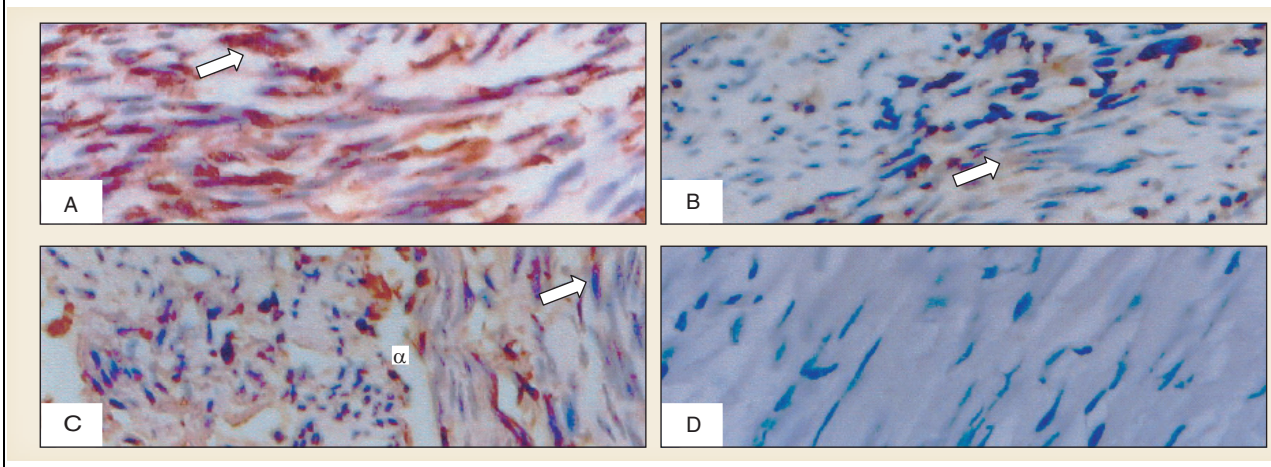
as leiomyomas at mid/late LP (increased estrogen/progesterone activity) and after LHRH-A therapy (minimal sex steroid hormone activity) contained similar TGFβ1 expression.

Previously, LHRH-A therapy was shown to alter Smads expression in human leiomyomas and myometrial tissues, thereby interrupting TGFβ.R signaling in these tissues [25]. Molecular studies targeting TGFβ1 activity showed that LHRH-A therapy resulted in selective regulation (differential regulation) of Smads in human uterine leiomyomas. Interestingly, the level of activated (phosphorylated) Smad-3, which was previously elevated in leiomyomas, was reduced by LHRH-A therapy [25]. These data have stressed the importance of TGFβ1 signaling-activity in the pathophysiology of uterine leiomyomas and the role of reduced Smad-3 activation as tissue-specific response to LHRH-A therapy.

However, since the uterine tissues contained specific LHRH.R, which mediates direct LHRH-A.R actions on leiomyomas, the question is whether the molecular changes in leiomyomas are caused by the reduction of sex steroid hormones and its direct or indirect molecular consequences, by the direct action of LHRH-A signaling pathway, or synergy of both these molecular events. Indeed, the LHRH-A signaling pathway can directly alter Smad-7 activity, an inhibitory Smad, which antagonizes TGFβ1 action on myometrial tissues via its interaction with TGFβ.R, thereby preventing the receptor-mediated activation of Smad-3 [26]. In addition, TGFβ1 signaling can activate other intracellular signal transduction pathways, including MAPK/ERK, which in turn can interact with sex steroid hormone signal transduction pathways and the LHRH-A signaling pathway at different levels [27–33]. Therefore, it is fair to conclude that complex molecular events, which implicate direct and indirect (cross talking) of various signal transduction pathways (generated by sex steroid ablation therapy plus LHRH.R signaling) mediate the clinical response of leiomyomas to LHRH-A therapy.

In concert with the above, microarray analysis documented that the expression profile of many

Figure 6. Increased immunochemical detection of TGFβ1 expression in leiomyomas.



An example of the increased immunochemical detection of the transforming growth factor beta 1 (TGFβ1) expression in leiomyomas at mid/late follicular phase **(A)** as compared with leiomyomas after luteinizing-hormone releasing-hormone (LHRH-A) therapy **(B)**, and to myometrium of women postmenopause **(C)**. **D:** The negative control of the immunohistochemical detection (without anti-TGFβ1 antibody).

altered genes in human uterine leiomyomas, including TGFβ1, TGFβ.R and Smad-3 overexpression, becomes similar to that of adjacent myometrium after LHRH-A therapy [25]. Consequently, our data concur with these findings since we detect similar TGFβ1 expression in leiomyomas as in the adjacent myometrium after LHRH-A therapy.

It is noteworthy that our data showed that the TGFβ1 expression did not differ significantly among myometrium at mid/late FP (increasing estrogen levels/absence of progesterone), myometrium at mid/late FP (increased estrogen/progesterone levels), myometrium postmenopause (minimal sex steroid hormone levels), suggesting that sex steroid ablation does not influence significantly TGFβ1 expression in normal myometrium. However, we should point out that hormonal influences can change pH and protease activity and consequently can affect indirectly the bioactivity of TGFβ1 locally [11]. In addition, diverse expression of other bioregulators among leiomyomas, such as plasminogen activator inhibitor (PAI)-1 and CUTL1, which can be caused by loss of the heterozygosity-reduced expression and chromosomal deletion contributes to diverse biology and clinical response of leiomyomas to LHRH-A therapy [20,23]. Therefore, the enhanced TGFβ1 expression in leiomyomas at mid/late FP is possibly attributed to complex tissue-specific interactions, regulating leiomyoma growth. These interactions may play a significant role in the pathophysiology of leiomyomas especially during this particular phase of the menstrual cycle.

Since under our cell culture conditions TGFβ1 (0.5 up to 50 ng/ml; final concentration) inhibited the growth of KW smooth muscle-like myometrial cells, we can assume that overexpression of TGFβ1 represents possibly a self attenuating tissue-specific response of leiomyomas to the unopposed (absence of progesterone) and progressively increasing concentration of estrogens (proliferative activity), occurring at mid/late FP of the menstrual cycle.

Highlights

- Transforming growth factor (TGF)β expression is higher in leiomyomas at mid/late follicular phase compared with those at luteal phase, and to adjacent myometrium at either phase of the menstrual cycle.
- As TGFβ inhibited the growth of KW smooth muscle-like myometrial cells, we assume that its overexpression represents a self-attenuating tissue-specific response of leiomyomas to the unopposed and progressively increasing concentration of estrogens, occurring at mid/late folliular phase of the menstrual cycle.

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