Thioredoxin expression in human myometrium and fibroids

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Thioredoxin is a small multifunctional protein which acts as a dithiol hydrogen donor for ribonucleotide reductase in DNA synthesis. Thioredoxin participates in the regulation of different metabolic processes, such as changes in the activity of different enzymes, receptors or transcription factors. The aim of the present study was to determine possible differences in the expression of thioredoxin between myometrium and fibroids in women during different periods of life. Thioredoxin mRNA concentrations were determined in myometrial and fibroid tissues obtained from women during the menstrual cycle, during treatment with an analogue of gonadotrophin releasing hormone (GnRH agonist), in the postmenopausal period (PMP) and during pregnancy. The concentration of thioredoxin mRNA was measured by a solution hybridization method. The localization of thioredoxin protein was examined by immunohistochemistry. There were significantly lower levels of thioredoxin expression in both fibroids and myometrium from GnRH agonist treated and PMP women in comparison with the pregnant women. No difference in thioredoxin expression was found between myometrium and fibroids from the same woman or between myometria from uteri with or without fibroids in the same patient group. Thioredoxin expression in uterine fibroids does not seem to be up-regulated, but changes in response to the endocrine conditions in a similar way to that observed in the myometrium.

Key words: fibroids/GnRH agonist/redox regulation/thioredoxin uterus

Introduction

Uterine fibroids (leiomyomas) are the most common tumours in women of reproductive age. They are composed of both muscle cells and fibroblasts, are benign and are of monoclonal origin (Mashal et al., 1994). The pathogenesis of uterine fibroids is not well understood, but may be due to hormonal imbalances and/or primary changes in the target cells. Fibroids do not occur before menarche and shrink after menopause, indicating that these tumours are sex steroid dependent. During the first trimester of pregnancy most fibroids grow, while during the third trimester a reduction in size often becomes evident (Lev-Toaff et al., 1987). It is notable that the decreased steroid receptor concentrations seen in the uterus during the second half of pregnancy mirror this decrease of fibroid growth (Khan-Dawood and Dawood, 1984; Padayachi et al., 1990). It has been shown that the concentrations of oestrogen and progesterone receptors are higher in the fibroids than in the surrounding myometrium in women of fertile age (Viville et al., 1997; Englund et al., 1998). For the mRNA concentrations of the progesterone receptor no difference was found between myometrium and leiomyomata, which implies that the difference in immunoreactive PR may result from post-translational control (Viville et al., 1997).

Treatment with an analogue to gonadotrophin-releasing hormone (GnRH agonist) causes decreased serum concentrations of oestrogens, progesterone, growth hormone and insulin-like growth factor-I (IGF-I) (Maheux and Lemay, 1988; Friedman et al., 1990) as well as an ~50% reduction of fibroid volume during a 3 month period (Healy, 1991). The antiprogestin (RU486) has proved to be as effective as GnRH agonist in reducing the size of fibroids after 3 months therapy (Murphy et al., 1993).

Thioredoxin is a multifunctional protein disulphide reductase (12 kDa) which plays a key role in many redox reactions, apart from its original function to supply electrons in the synthesis of deoxyribonucleotides by ribonucleotide reductase, which is essential for DNA replication (Holmgren, 1985; Holmgren and Björnstedt, 1995). Thus, other functions for thioredoxin are to regulate the activity of enzymes and transcription factors by thiol redox control or to participate in defence against reactive oxygen species either directly or as an electron donor for thioredoxin peroxidases (Zhang et al., 1997; Holmgren et al., 1998). Thioredoxin is secreted from cells and operates in synergy with cytokines to stimulate cell growth and is able to activate protein kinase C (Rubartelli et al., 1992; Biguet et al., 1994; Rosén et al., 1995; Di Trapani et al., 1998).

Thioredoxin has been demonstrated in human decidua and trophoblasts, where it was suggested to protect the fertilized egg and placental trophoblasts from the cytotoxic effects of
oxygen radicals (Kobayashi et al., 1995; Perkins et al., 1995; DiTrapani et al., 1998). In humans, thioredoxin expression in the endometrium was shown to be highest in the early secretory phase of the menstrual cycle (Maruyama et al., 1997). This period is influenced by the oestrogen peak prior to ovulation, but also by increasing serum concentrations of progesterone. It is difficult to distinguish between the influence of oestrogen and progesterone in human tissue samples, since both hormones are present. Therefore we have previously studied the effects of hormone treatment in the uterus in a rat model system, since ovariectomy can be used as a tool to limit endogenous hormone exposure. We found that oestradiol, testosterone and 5α-dihydrotestosterone increased the thioredoxin mRNA concentration in the rat uterus whereas progesterone, dexamethasone and growth hormone did not affect thioredoxin expression (Sahlin et al., 1997a, 1999). None of these hormones increased thioredoxin mRNA concentrations in the livers of the same animals (Sahlin et al., 1997a, 1999). The oestrogen-mediated increase of the thioredoxin mRNA concentrations was attenuated by simultaneous treatment with the anti-oestrogen ICI 182780, whereas the androgen-mediated increase was attenuated by the anti-androgen Flutamid (Sahlin et al., 1999). Thus, in rats the uterine thioredoxin mRNA concentration has been shown to be regulated, at least partly, by growth-promoting gonadal steroids while the hepatic concentration was unchanged, implicating a tissue-specific regulation. In human cervical stroma from non-pregnant women, we found a positive correlation between the concentrations of thioredoxin mRNA and serum oestradiol, but no correlation with the serum progesterone (Sahlin et al., 1997b).

Recently a study on thioredoxin expression in cultured stromal cells from human endometrium showed that both thioredoxin and its mRNA was up-regulated after incubation with oestradiol, whereas the expression was suppressed by the oestrogen antagonist tamoxifen (Maruyama et al., 1999). In addition, epidermal growth factor-dependent mitogenesis was additively enhanced by thioredoxin treatment, although thioredoxin had no effect on growth promotion of the stromal cells on its own (Maruyama et al., 1999).

The aim of the present study was to investigate whether the expression of thioredoxin, which seems to correlate with growth induction in the rat uterus and in human endometrium and cervical stroma, is changed in myometrial fibroids as compared to the surrounding myometrium, thus offering an explanation to the selective growth advantage of these tumours.

**Materials and methods**

**Patients**

Thirty-nine women with fibroid disease, admitted for myomectomy or hysterectomy, were included in this study. The clinical diagnosis was based on pelvic examination and an ultrasound scan. The indication for surgery was one or more of the following: menorrhagia, dysmenorrhoea, pelvic discomfort, rapid tumour growth, and uncertainty about tumour diagnosis.

Five women were postmenopausal (PMP). They had not been menstruating for at least 6–12 months prior to surgery. They had not received hormone replacement therapy during the last 6 months.

Five patients with fibroids were pregnant and the samples were collected from four of them during elective Caesarean sections at term pregnancy. From two of the pregnant women with fibroid disease only fibroid tissue, and from another woman only myometrium, were obtained. One woman, 46 years of age, underwent hysterectomy in the 12th week of pregnancy, due to a wish for an abortion and multiple large fibroids making vacuum aspiration impossible.

Thirteen of the patients were offered and received pre-operative treatment with a GnRH analogue (goserelin) in order to reduce the symptoms and uterine size before surgery. This treatment was given 2–3 months before admission. The dose of goserelin was 3.6 mg s.c. every 4 weeks. From three of the GnRH agonist-treated women only fibroid tissue was obtained.

The menstruating women were subclassified into 'proliferative' and 'secretory' groups according to the phase of the menstrual cycle. This classification was based on the menstrual cycle pattern, the last menstrual period and serum concentrations of oestradiol and progesterone (Table I). None of these patients was given any hormonal treatment prior to surgery. Several untreated women could not be classified into the above categories because of menstrual irregularity, hormone concentrations indicating a postmenopausal state, or both. These women, as well as those with adenomyosis, were not included in the present study.

Control myometrium from 17 women without any history of uterine disorders were obtained, and divided into the following groups: proliferative phase (n = 6), PMP (n = 5) and pregnant (n = 6) (Table II).

**Tissue collection**

All operations, except the Caesarean sections done at term and performed with spinal anaesthesia, were performed under general anaesthesia.

Tissues were collected from fibroids and myometrium immediately after removal of the uterus or fibroid, or per operatively. In the case of multiple fibroids, specimens were collected from the largest tumours. The specimens were either immediately frozen in liquid nitrogen and stored at ~70°C until analysis or fixed in 10% formaldehyde in methanol, and subsequently embedded in paraffin for future microscopical analyses.

**Preparation of total nucleic acids**

Total nucleic acids (TNA) were prepared by digestion of homogenized tissue with proteinase K in a sodium dodecyl sulphate-containing buffer, followed by subsequent extraction with phenol–chloroform as described before (Sahlin, 1995). The concentration of DNA in the TNA samples was measured fluorometrically at the wavelength 458 nm with Hoechst Dye 33258 (Labarca and Paigen, 1980).

**Hybridization probes**

The probe used for the thioredoxin mRNA determinations was derived from a clone of human thioredoxin cDNA as previously described (Lippoldt et al., 1995). A fragment of 315 base pairs representing the 105 amino acids in the open-reading frame of the human thioredoxin gene was subcloned into a pGEM 3Z vector and used as described previously (Sahlin et al., 1997b). For solution hybridization the probe was labelled with 35S-UTP. A Northern blot showing the hybridization of human uterine RNA with this probe was presented in Sahlin et al. (1997b). The mRNA has the size of ~0.6 kb.

**Hybridization analysis of mRNA**

A solution hybridization assay of specific mRNA was used and performed as presented before (Sahlin, 1995). The method was modified to allow quantitative measurement of mRNA, as follows.
High-resolution pass

Table I. A. Patient characteristics and hormone concentrations in serum. Means and ranges are given.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Age (years)</th>
<th>Parity</th>
<th>Oestradiol (pmol/l)</th>
<th>Progesterone (nmol/l)</th>
<th>Testosterone (nmol/l)</th>
<th>FSH (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative phase</td>
<td>42.7 ± 2.6</td>
<td>1.4</td>
<td>718 ± 62</td>
<td>1.6 ± 0.6</td>
<td>1.7 (6)</td>
<td>n.d.</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>35–48</td>
<td>(0–3)</td>
<td>240–1596</td>
<td>0.5–4.1</td>
<td>1.0–2.4</td>
<td></td>
</tr>
<tr>
<td>Secretory phase</td>
<td>44.8 ± 2.8</td>
<td>1.8</td>
<td>402 ± 65</td>
<td>26.1 ± 4.5</td>
<td>1.7 (3)</td>
<td>n.d.</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>39–50</td>
<td>(0–3)</td>
<td>225–765</td>
<td>5.5–45.3</td>
<td>1.4–1.9</td>
<td></td>
</tr>
<tr>
<td>Pregnant (n = 4)</td>
<td>36.8 ± 2.0</td>
<td>1.2</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>(34–46)</td>
<td>36.0 ± 0.9</td>
<td>0.9</td>
<td>59.5 ± 6.4</td>
<td>0.9 ± 0.4</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>GnRHa</td>
<td>64 ± 6.6</td>
<td>1.4</td>
<td>106 ± 10</td>
<td>1.1 ± 0.1</td>
<td>1.5 ± 0.5</td>
<td>35.4 ± 5.4</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>53–69</td>
<td>(0–3)</td>
<td>25.0–195</td>
<td>0.4–2.9</td>
<td>1.2–1.8</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal period (n = 5)</td>
<td>64 ± 6.6</td>
<td>1.4</td>
<td>106 ± 10</td>
<td>1.1 ± 0.1</td>
<td>1.5 ± 0.5</td>
<td>35.4 ± 5.4</td>
</tr>
<tr>
<td></td>
<td>53–69</td>
<td>(0–3)</td>
<td>25.0–195</td>
<td>0.4–2.9</td>
<td>1.2–1.8</td>
<td></td>
</tr>
</tbody>
</table>

*For one of the postmenopausal control patients no blood sample was collected.
FSH = follicle stimulating hormone; GnRHa = gonadotrophin releasing hormone agonist; n.d. = not determined.

Table I. B. Control myometrium. Patient characteristics and hormone concentrations in serum

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Age (years)</th>
<th>Parity</th>
<th>Oestradiol (pmol/l)</th>
<th>Progesterone (nmol/l)</th>
<th>FSH (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative phase</td>
<td>44.5 ± 2.6</td>
<td>1.7</td>
<td>1121 ± 87</td>
<td>1.5 ± 0.5</td>
<td>n.d.</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>35–50</td>
<td>(0–3)</td>
<td>149–4540</td>
<td>0.5–3.1</td>
<td></td>
</tr>
<tr>
<td>Pregnant phase</td>
<td>30 ± 2.0</td>
<td>1.8</td>
<td>&gt;14 000</td>
<td>&gt;150 ± 10</td>
<td>n.d.</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>24–38</td>
<td>(1–3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>69 ± 6.6</td>
<td>2.0</td>
<td>74.7 ± 6.4</td>
<td>0.8 ± 0.2</td>
<td>48.9 ± 3.6</td>
</tr>
<tr>
<td>period (n = 4)</td>
<td>54–84</td>
<td>(0–4)</td>
<td>69–134</td>
<td>(0.2–1.1)</td>
<td>(36.9–54.6)</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

The results are given as mean ± SEM.

Table II. Thioredoxin mRNA concentrations in normal myometrium from women without any uterine disorder

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Thioredoxin mRNA (attomol/µg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative phase</td>
<td>1.26 ± 0.18 ± 0.18</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
</tr>
<tr>
<td>Pregnant (n = 6)</td>
<td>12.0 ± 1.66 ± 0.15</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>0.85 ± 0.15 ± 0.15</td>
</tr>
</tbody>
</table>

The results are given as mean ± SEM.

The radioactivity of the sample was compared with a standard curve of known amounts of mRNA synthesized in vitro and complementary to the probe used. Results were expressed as attomol (10^-18)/µg DNA in the TNA samples.

**Hormone determinations**

Blood samples were collected between 0700 and 0800 on the morning of surgery. Serum concentrations of 17β-oestradiol, progesterone and follicular stimulating hormone were determined by enhanced luminescence competitive immunoassay using commercial kits (Amerlite) obtained from Amersham International, Little Chalfont, Bucks, UK. Serum follicle stimulating hormone (FSH) concentrations are expressed as IU/l of the 2nd International Reference Preparation 78/549. Detection limits and within- and between-assay coefficients were for oestradiol 50 pmol/l, 13.3% and 14.8%, for progesterone 35 nmol/l, 11.1% and 16.9% and for FSH 0.5 IU/l, 5% and 7.6%, respectively. In the statistical calculations values below the detection limits were set to 25 pmol/l for oestradiol and to 0.20 nmol/l for progesterone respectively. Serum concentrations of testosterone were determined by a 3H radioimmunoassay method, using a tracer from DuPont, NEN ([1,2,6,7- 3H]testosterone) and antiserum (testosterone AB) from ICN Biomedicals Inc., Costa Mesa, CA, USA. Within- and between-assay coefficients were 5% and 8% respectively. In the statistical calculations values below the detection limits were set to 0.1 nmol/l.

**Immunohistochemistry**

Paraffin sections (5 µm) from myometrium and fibroids were used to illustrate the distribution of thioredoxin in each group of patients (GnRH agonist, PMP, P-phase, S-phase and Preg). A standard immunohistochemical technique (avidin–biotin–peroxidase) was used to visualize the thioredoxin immunostaining. Human recombinant thioredoxin was expressed and purified to homogeneity as described previously (Ren et al., 1993). Polyclonal anti-human antisera were obtained by immunization of a goat with oxidized thioredoxin by the technique previously described (Rozell et al., 1985) and pure antibodies were obtained by affinity chromatography on immobilized human thioredoxin by the method developed for E.coli thioredoxin (Nordström et al., 1981).

Immunohistochemistry procedures were similar to those previously described (Wang et al., 1999). Negative controls were obtained by replacing the primary antibody with non-immunoserum of the equivalent concentration. In addition, the thioredoxin antibody was pre-absorbed with thioredoxin overnight to demonstrate antigen specificity. Following primary antibody binding, the sections were incubated for 60 min at RT with a biotinylated rabbit anti-goat IgG antibody (Vector, Burlingame, CA, USA), diluted in normal rabbit serum. The tissue sections were incubated for 60 min at RT with horseradish peroxidase–avidin–biotin complex (ABC-Elite; Vector).
The site of the bound enzyme was visualized by the application of 3,3′-diaminobenzidine in H₂O₂ (DAB kit; Vector), a chromogen which produces a brown, insoluble precipitate when incubated with enzyme. The sections were counterstained with haematoxylin and dehydrated before mounting with Pertex.

A Leica microscope connected to a video camera (Sony) and computer was used to assess immunostaining photographs.

Statistics
Values are given as mean ± SEM. Statistical calculations were performed with the Kruskal–Wallis test, significance was evaluated with Dunn’s test and P < 0.05 was considered to be significant. Correlation analyses were performed with Spearman’s rank test. Values with different letter designations are significantly different.

Ethics
The design of the study was approved by the Ethics Committee of the Huddinge University Hospital and the corresponding committee of the Uppsala University Hospital. Informed consent was obtained from all participating women.

Results
No differences were found in the thioredoxin mRNA concentrations between fibroids and myometrium in any of the patient groups (Figure 1). There was a significant difference in the thioredoxin mRNA concentration between the myometrium of pregnant women and those of GnRH agonist-treated and PMP women (Figure 1; P < 0.05). The thioredoxin mRNA concentration in control myometrium from women without fibroids was higher in pregnant women than in the proliferative phase group or the PMP women (Table II). The thioredoxin mRNA concentration in myometrium from pregnant women without fibroids (12.0 attomol/µg DNA ± 1.66) was higher than that of the myometrium from pregnant women with fibroids (7.38 ± 1.54) but not to a degree statistically significant, possibly due to the limited number of observations in the latter group (n = 3). The thioredoxin mRNA concentration was lower in fibroids from GnRH agonist and PMP patients than in fibroids from pregnant women (Figure 1). In fibroids from GnRH agonist patients the thioredoxin mRNA concentration was lower than in fibroids from women in the secretory phase of the menstrual cycle (Figure 1). There was a positive correlation between the thioredoxin mRNA concentration in the fibroids of PMP women and their serum oestradiol concentration (r = 0.9, n = 5, P = 0.037). There was also a positive correlation between the thioredoxin mRNA concentration in the fibroids of women in the proliferative phase and their serum testosterone concentration (r = 0.89, n = 6, P = 0.019). No other parameters in the different groups showed significant correlation (data not shown).

The thioredoxin mRNA concentration was higher in the myometrium and fibroids of pregnant women as compared to the other patient groups (8–12 versus 0.8–2.5 amol/µg DNA). This could also be shown for the protein itself by immunohistochemistry, where positive immunostaining was found both in the nuclei and cytoplasm (Figure 2). The negative control incubated with the thioredoxin peptide prior to immunolabelling showed no immunostaining (Figure 2A). The immunoreactivity of thioredoxin (brown colour) was highest in the fibroid from a pregnant woman (Figure 2B). The myometrium and fibroids from women in proliferative (Figure 2C and D) and secretory (Figure 2E and F) phases showed less immunostaining than the fibroid from a pregnant woman (Figure 2A). The least positive immunostaining was found in the myometrium and fibroids of GnRH agonist-
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Figure 2. Immunohistochemical localization of thioredoxin in uterine tissues. (A) Negative control, thioredoxin peptide incubated with the antibody prior to tissue incubation. No positive staining detectable. (B) Fibroid tissue from a term pregnant patient. (C), (E), (G), and (I) are representative images of myometrium from women in proliferative phase, secretory phase, after gonadotrophin releasing hormone agonist treatment and postmenopausally, respectively. (D), (F), (H), and (J) are images of thioredoxin staining in fibroids from the same patients as above. In (F) and (G), the V denotes vessels. Scale bar = 30 µm.

treated (Figure 2G and H) and PMP women (Figure 2I and J). Thus, the protein and mRNA concentrations were in agreement in all categories (Figures 1 and 2). It is notable that the positive immunostaining for thioredoxin was translocated into the nucleus with increasing concentrations of oestradiol in the serum, i.e. the pregnant group (B).
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Discussion

The present findings indicate that, in myomas and myometrium, the concentration of the regulatory molecule thioredoxin is related to the endocrine condition of the women. Furthermore, no tissue differences in thioredoxin expression were observed between myomas and normal myometrium in any of the different groups of patients. Consequently, it is reasonable to assume that sex steroid-dependent expression of thioredoxin does not contribute to the selective growth advantage of leiomyomas in women of fertile age.

In the present work we found a positive correlation between the serum oestradiol concentration and the thioredoxin mRNA concentration in the fibroids from PMP women, with low circulating oestrogen concentrations in serum. In addition, in fibroids from women in the proliferative phase of the menstrual cycle, the thioredoxin mRNA concentrations correlated to the serum testosterone concentrations. These interesting findings are in agreement with our results from ovariectomized rats, where oestradiol and androgens increased uterine thioredoxin mRNA concentrations (Sahlin et al., 1997a, 1999).

In the cervical stroma from non-pregnant women the thioredoxin mRNA concentration is positively correlated with the serum oestradiol concentration (Sahlin et al., 1997b). In the cervix of pregnant women the thioredoxin mRNA concentration is ~4-fold higher than in the cervix of non-pregnant women. No correlation with the serum oestradiol concentration was observed, probably due to the very high oestrogen concentrations at the end of pregnancy resulting in an already maximal expression of thioredoxin (Sahlin et al., 1997b).

In the uterus from ovariectomized rats the thioredoxin mRNA concentration is increased after treatment with certain gonadal steroids. Interestingly, it is only the gonadal steroids that cause induction of growth in the uterus, which stimulate an increase of the thioredoxin mRNA concentration in the rat. Progesterone on its own induces neither uterine growth nor expression of thioredoxin in the rat uterus (Sahlin et al., 1999).

Thiol redox control via thioredoxin (Holmgren, 1985) is of growing interest for its importance in regulating the binding of transcription factors to DNA (Schenk et al., 1994; Sen and Packer, 1996). The transcription factors nuclear factor-kB (NF-kB) and activator protein-1 (AP-1) are regulated by the intracellular redox state (Sen and Packer, 1996). The AP-1 binding site is present in the promoter region of genes associated with growth, transformation and differentiation (Karin, 1991). The thioredoxin system has been demonstrated to stimulate DNA-binding of the transcription factor AP-1 (Schenk et al., 1994). AP-1 is an important mediator of tumour promotion (Angel and Karin, 1991; Karin, 1995). Interaction between c-Jun and c-Fos proteins (products of the c-fos and c-fos protooncogenes) has been shown to have important regulatory implications on the expression of growth factor-inducible genes (Angel and Karin, 1991). c-Fos protein and mRNA have shorter half-lives than cJun protein and mRNA. Before induction (with 12-O-tetradecanoylphorbol 13-acetate) most of the AP-1 complex is present in the form of Jun homo- and heterodimers, whereas after induction Fos proteins are expressed and most of the AP-1 complex is present as Fos–Jun heterodimers (Sen and Packer, 1996). It has been shown that thioredoxin is translocated from the cytosol to the nuclei regulating transcription factor activity (Hirota et al., 1997). Thioredoxin also interacts with an intranuclear reducing molecule, Ref-1, which modifies the DNA-binding activity of AP-1 (Hirota et al., 1997). Ref-1 was found in both myometrium and leiomyomas (Nikaido et al., 1999). Thus, regulation of the activity of transcription factors may be an important general mechanism by which the thioredoxin system participates in many different cellular processes.

A recent report showed that c-Jun expression in myometrium from pregnant women without fibroids was higher than in myometrium from the corresponding non-pregnant women, indicating that the gene responds to excessive sex steroid stimulation (Gustavsson et al., 1999). Moreover, the c-fos mRNA concentration in myometrium from patients with fibroid disease was not different from that in myometrium of control patients under similar endocrine conditions (Gustavsson et al., 1999). This concept is in agreement with studies in the mouse, demonstrating that administration of oestradiol to adult ovariectomized animals caused differential c-jun expression in all uterine cell types, whereas c-fos was not detected in the stromal and myometrial cells (Yamashita et al., 1996). These findings suggest that thioredoxin stimulates the DNA-binding of the transcription factor AP-1 (Schenk et al., 1994; Hirota et al., 1997) by stimulation of c-jun expression in the myometrium, and that this regulation is related to the endocrine condition of the woman and not to specific changes in pathological tissue. Oestrogen hormones have been shown to induce transient transcriptional activation of c-fos, mediated via a functional oestrogen responsive element (ERE) in the human c-fos gene (Weisz and Rosales, 1990; Ambrosino et al., 1993). It has also been shown that Fos overexpression in HeLa cells induces significant reduction in the activity of oestrogen receptor-mediated activation of the c-fos gene (Ambrosino et al., 1993). Thus, there is a functional interference between the ER and Fos in the regulation of c-fos proto-oncogene transcription (Ambrosino et al., 1993).

It is notable that the immunostaining of thioredoxin is predominantly in the cytosol in the tissues from women with the lower oestradiol concentrations (e.g. GnRH agonist). With increasing oestradiol concentrations the positive staining is also located in the nuclei of the cells (e.g. pregnant). This indicates that oestrogens could stimulate the translocation of thioredoxin in the human sponge Geodia cydonium (Wiens et al., 1999) and in this species no ER has been detected. It was concluded that oestradiol displayed its effect via production of reactive oxygen species (ROS). At physiological doses ROS causes the increased expression of thioredoxin, at higher doses the sponge cells undergo apoptosis (Wiens et al., 1999). Thioredoxin is overexpressed in some human tumours (Powis et al., 1997), and also secreted by tumour cells, enhancing the...
sensitivity of cancer cells to other growth factors (Schenk et al., 1996).

Taken together, our data suggest that thioredoxin is not part of the altered fibroid phenotype responsive for the selective growth advantage of this benign neoplasm. We suggest that thioredoxin is part of the complex cascade of events involved in the regulation of uterine growth and metabolism, following exposure to and withdrawal of sex steroid hormones. Increased knowledge about these mechanisms may have strong implications for development of new medical regimens for modulating myoma size.

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