Hormonal Regulation of Catechol-O-Methyltransferase Activity in Uterine Leiomyoma

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"Old Red"
Built in 1891

ABSTRACT

Objectives: Catechol-O-methyltransferase (COMT) plays an important role in estrogen metabolism. COMT converts catechol estrogens into their methoxy derivatives. Two promoters control the expression of human COMT isoforms: membrane-bound COMT and soluble COMT, which are under the control of two distinct promoters (COMTP2 and COMTP1, respectively). Regulation of human COMT gene expression may be important in the pathophysiology of various estrogen-related disorders.

Methods and Results: RNA microarray analysis indicated that COMT exhibits about 3.4-fold higher expression in leiomyoma tumor tissues compared with the adjacent normal myometrium. Immunohistochemical staining with anti-COMT specific polyclonal antibodies confirmed stronger COMT protein expression in leiomyomatous tumor tissues compared to normal myometrial tissue from the same uterus. Analysis of COMTP1 and COMTP2 indicated that both promoters harbor half sites of estrogen response element (TGACCT) and glucocorticoid response element (TGTTCT), suggesting that these promoters might be regulated by estrogen and glucocorticoids. Indeed, treatment of ELT-3 rat leiomyoma cells with 17-ß-estradiol (10^{-9}–10^{-6} M) resulted in downregulation of COMT protein expression in Western blot analysis. Similarly, in ELT-3 cells cotransfected with COMT promoter-luciferase reporter, 17-ß-estradiol downregulated both COMTP1- and COMTP2-luciferase activities in a dose-dependent manner. Contrary to estradiol, progesterone and dexamethasone (10^{-10}–10^{-6} M) upregulated COMT protein expression and COMTP1-and COMTP-luciferase activities in a dose-dependent manner.

Conclusion: Our findings present a molecular mechanism for the interactive effect of different steroid hormones in the pathophysiology of uterine leiomyoma.

INTRODUCTION

Uterine leiomyomas (ULM) are the most common pelvic tumors in the United States, occurring in 20% to 25% of premenopausal women. Epidemiological and experimental animal studies have established a role for ovarian hormones in the pathogenesis of ULM. Although clinical and biochemical observations have traditionally supported an important role for estrogen in the promotion of ULM growth, there is also increasing evidence to suggest the involvement of progesterone. Indeed, the exact mechanism by which these ovarian steroid hormones initiate/promote ULM is still incompletely revealed. In fact, estrogen metabolites play a critical role in many estrogen-related tumors. Earlier studies suggested that endogenous levels of catechol estrogens are significantly different between ULM and adjacent normal myometrium. Consequently, it is conceivable to expect that regulation of estrogen metabolizing genes might have an etiological role in the development of ULM. Catechol-O-methyltransferase (COMT) metabolizes many endobiotics, including catechol estrogens. Therefore, regulation of COMT activity may indirectly modulate the biological effects of estrogen, and may play an etiologic role in leiomyoma formation.

AIM

The current study was undertaken to investigate the expression of COMT in leiomyoma tissues and normal adjacent myometrium. Also, our study aimed at investigating the regulation of COMT expression by estrogen, progesterone, and dexamethasone in the ELT-3 rat leiomyoma cell line.

MATERIALS & METHODS

Patient selection and human tissue processing

Patients presenting with symptomatic uterine fibroids requesting definitive treatment in the form of hysterectomy were recruited for the study. Tissues (leiomyoma and normal myometrium) were collected. Patients did not receive any hormonal or GnRH agonist treatment prior to surgery. Tissue samples were submersed immediately in liquid nitrogen or formalin and transferred to the research area. RNA and protein were isolated for microarray and Western blot analysis.

Immunohistochemistry

Human tissue specimens were collected from representative areas and fixed in buffered formalin. Paraffin-embedded tissue sections (5 µm) were deparaffinized and rehydrated by passage through xylene and graded ethanol solutions. Primary anti-COMT polyclonal antibody raised in guinea pig was applied to sections at a 1:250 dilution.

Construction of chimeric COMT proximal promoter and COMT distal promoter luciferase reporters

COMT proximal promoter (COMTP1) was amplified by polymerase chain reaction (PCR) using genomic DNA isolated from normal human myometrial tissues as a template, and with the following primers: forward primer (fP1) *5′-GATCGCGT* ACATCTGCTTTGCTGCCGA-3 (containing *Mlu*I recognition sites at the 5′ end, the italic letters) and the reverse primer (rP1); *5′- GGAAGATCT* GAGCAGGTTGTGGATGGG-3 (containing the recognition sites for *Bgl* II restriction enzymes at the 5′ end, italic letters). For COMT Distal Promoter (COMTP2), the following primers were used: forward primer (fP2); *5′- GATCGCGT* GCTCCTCTGGCGGAAAGGA-3 (containing *Mlu*I recognition sites at the 5′ end, the italic letters) and reverse primer (rP2): *5′-GGAAGATCT* CTCTCCCGCGACGGCCCG-3 (containing the recognition sites for *Bgl* II restriction enzymes at the 5′ end, italic letters). The PCR products were then purified and cloned into pGEM-T easy cloning vector. The cloned COMTP1 and COMTP2 were released from the pGEM-T easy vector by digestion with *Mlu*I and *Bgl*II enzymes and subcloning into *Mlu*I and *Bgl* II linearized PGL3 luciferase reporter vector. The identity of the cloned insets was confirmed by sequencing and comparison with published sequences. Colonies containing plasmids with the correct inserts were cultured, and plasmid DNA was isolated using QIAFilter (Qiagen Inc, Valencia, Calif) plasmid midi kits according to manufacturer's protocol.

Cells and cell culture

ELT-3 cells were maintained at 37°C in 5% CO_2 /air in Dulbecco's modified Eagle's medium (DMEM), with 10% fetal bovine serum. Cells were grown in phenol red-free DMEM supplemented with 10% calf serum depleted of endogenous estrogen, antibiotics (100 U/mL of penicillin and 100 µg/mL of streptomycin), and 2-mM L-glutamine. Cells (2×10^6) were seeded onto 100-mm culture dishes and incubated 24 hours. The media were removed, and the cells were reincubated in fresh media with different concentrations of E_2 (10^{-10}–10^{-6} M) in 0.01% (v/v) ethanol, or without E_2 (control) for 72 hours. For studying the effect of progesterone and dexamethasone, ELT-3 cells were grown in 90% phenol red-free DMEM supplemented with 10% charcoal-stripped calf serum and supplemented with 10^{-8} M exogenous estrogen. The cells were treated with different concentrations (10^{-9}–10^{-6} M) of progesterone, or (10^{-9}–10^{-6} M) of dexamethasone in 0.01% (v/v) ethanol, or only 0.01% ethanol alone (as control) for 72 hours. The cells were then harvested for protein assay.

Mammalian cell transfection with luciferase or –galactosidase plasmids

The activities of COMTP1-Luc and COMTP2- Luc constructs were determined in transiently transfected ELT-3 cells. Cells (60%–70% confluent) were cotransfected with luciferase reporter constructs (10 µg), and Promega pSV-ß-galactosidase control vector (1 µg) using calcium phosphate transfection method. After incubation for 5 hours at 37°C, the medium was removed, and the cells were incubated in fresh medium with the addition of different concentrations of E_2 (10^{-9}–10^{-10} M) in 0.01% (v/v) ethanol, or without E_2 (control). For progesterone and dexamethasone, concentrations of 10^{-9}–10^{-6} M were used. After 48 hours incubation of the transfected cells with different treatments, luciferase and pSV-ß-galactosidase activities were determined using ß-galactosidase and luciferase assay Kits (Promega, Madison, Wis). Protein concentrations were determined by the BCA protein assay kit (Pierce Co. Rockford, Ill). Luciferase activity is expressed after normalization against ß-galactosidase activity and protein concentration.

SDS-PAGE/Western blot analysis

Western blot analyses were performed using whole cell homogenate (50 µg per lane) prepared from control (ethanol only), estradiol, progesterone, or dexamethasone-treated cells according to the standard protocol. Purified COMT antibody (1:1500) raised in sheep was used to detect the COMT protein. Membranes were developed using HRP-conjugated sheep IgG with ECL Western Blotting Detecting Reagents (Amersham Biosciences, UK). The intensity of each band was determined using a scanning densitometer (Epson 4870, Epson America, Long Beach, Calif)

RESULTS

Upregulation of COMT Gene Expression in Human Leiomyoma Specimens

Table 1. Fold change of the expression of catechol-O-methyl transferase gene (accession: M58525) in uterine leiomyoma relative to adjacent normal myometrium in array analysis

	Patient 1	Patient 2	Patient 3	Mean ± SEM
Large uterine leiomyoma vs normal myometrium	4.8	3.4	2.1	3.43 ± 0.78
Small uterine leiomyoma vs normal myometrium	1.7	2.9	1.6	2.07 ± 0.42

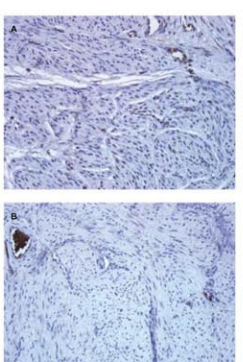

Figure 1. Immunohistochemical staining with COMT antiserum. There was more intense cytoplasmic COMT-specific staining in uterine leiomyoma (A) compared with adjacent normal myometrium (B) from the same patient. Arrow indicates deep staining of the COMT-rich red blood cells in a small blood vessel (×200).

Structural analysis of human COMTP1 and COMTP2 promoters

Figure 2. Nucleotide sequences and putative regulatory elements of human COMT P1 (A) and P2 (B). **A)** EREs and GREs are underlined. Translation start position for 1.5-kb transcript is at +1, and for 1.3-kb transcript is at +151 in exon 3. **B)** position of 5′-end of the longest 1.5-kb transcript is at +1 in exon 1.

Hormonal Regulation of COMT Protein Expression in Rat Leiomyoma Cells

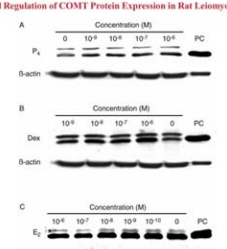

Figure 3. SDS-PAGE/Western blot analysis of MB-COMT and S-COMT protein level in ELT3 leiomyoma cells treated with different concentrations of progesterone (10^{-9}, 10^{-8}, 10^{-7}, or 10^{-6} M, **A**); different concentrations of dexamethasone (10^{-9},10^{-8}, 10^{-7}, or 10^{-6} M, **B**); or different concentrations of 17—estradiol (10^{-10},10^{-9}, 10^{-8}, 10^{-7}, or 10^{-6} M, C) for 72 h. MCF-7 breast cancer cell lysate was used as positive control. COMT polyclonal antibody was used at 1:1500. ß-actin was used as loading control.

Hormonal regulation of COMT promoter-reporter expression in ELT-3 cells

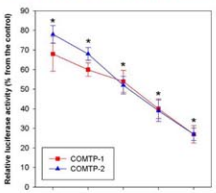

Figure 4A. Effect of 17—estradiol on luciferase activity in ELT-3 cells cotransfected by COMTP-Luc constructs and pSV-ß-galactosidase. Constructs were cotransfected into ELT-3 cells and incubated with E_2 (10^{-10}–10^{-6}M) or without E_2. After 48 hours, the cells were harvested. Luciferase and ß-galactosidase activities were determined using ß-galactosidase and luciferase assay kits (Promega, Madison, Wis) according to the manufacturer's protocol. The results were expressed as a percentage of luciferase activity in E_2-treated cells relative to non–E_2-treated cells after correction for transfection efficiency using ß-galactosidase and protein concentration. The mean values ± SEM of at least three separate transfections are shown.

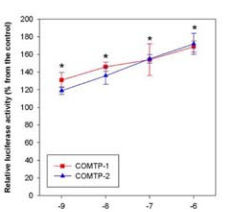

Figure 4B. Effect of progesterone on luciferase activity in ELT-3 cells cotransfected by COMTP-Luc constructs and pSV-ß-galactosidase. Constructs were cotransfected into ELT3 cells and incubated with Progesterone (10^{-9}–10^{-6} M) or without progesterone. After 48 hours, the cells were harvested. Luciferase and ß-galactosidase activities were determined using ß-galactosidase and luciferase assay Kits (Promega, Madison, Wis) according to the manufacturer's protocol. The results were expressed as a percentage of luciferase activity in progesterone-treated cells relative to non-treated control cells after correction for transfection efficiency using ß-galactosidase and protein concentration. The mean values ± SEM of at least three separate transfections are shown

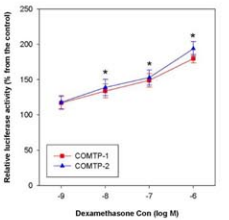

Figure 4C. Effect of dexamethasone on luciferase activity in ELT-3 cells cotransfected by COMTP-Luc constructs and pSV-ß-galactosidase. Constructs were cotransfected into ELT3 cells and incubated with dexamethasone (10^{-9}–10^{-6} M) or without dexamethasone. After 48 hours, the cells were harvested. Luciferase and ß-galactosidase activities were determined using ß-galactosidase and luciferase assay kits (Promega, Madison, Wis) according to the manufacturer's protocol. The results were expressed as a percentage of luciferase activity in dexamethasone-treated cells relative to nontreated control cells after correction for transfection efficiency using ß-galactosidase and protein concentration. The mean values ± SEM of at least three separate transfections are shown.

CONCLUSION

COMT is highly expressed in leiomyoma tissues compared to the normal myometrium. COMT transcription is under the control of steroid hormones. While estradiol suppresses expression of COMT, progesterone and dexamethasone upregulate COMT transcription and protein expression. Our data may provide a possible explanation for the role of COMT in the physiologic and pathologic conditions of women.