The role of TWIST, SERPINB5, and SERPIN1 genes in uterine leiomyomas

Mehmet Sühha Bostancı[1], Merih Bayram[2], Süleyman Murat Bakacak[3], Özge Kızılkale Yıldırım[4], Rukset Attar[4], Gazi Yıldırım[4], Emin Ümit Bağrıaçık[5], Baran Celtemen[2]

[1]Department of Obstetrics and Gynecology, Sakarya University Faculty of Medicine, Sakarya, Turkey
[2]Department of Obstetrics and Gynecology, Gazi University Faculty of Medicine, Ankara, Turkey
[3]Department of Obstetrics and Gynecology, Sütçü İmam University Faculty f Medicine, Kahramanmaraş, Turkey
[4]Department of Obstetrics and Gynecology, Yeditepe University Faculty of Medicine, İstanbul, Turkey
[5]Department of Immunology, Gazi University Faculty of Medicine, Ankara, Turkey

Abstract

Objective: The aim of this study is investigate the role of the Twist homolog 1 (TWIST), serine peptidase inhibitor (SERPINB5), and plasminogen activator inhibitor 1 (SERPIN1) genes in uterine leiomyoma etiopathogenesis.

Material and Methods: Twelve patients, aged between 39 and 58, and had a hysterectomy, were included in the study. The size of the leiomyomas was between 20 and 130 mm based on gross pathology after hysterectomy. Tissue samples were obtained from normal myometrium and leiomyoma (1 cm^3) tissue of the uterus of the patients and stored at -86°C. Samples were divided to two groups after histopathological evaluation of the uterus: normal myometrial tissues as control group (Group 1) and leiomyoma tissue as the study group (Group 2). The TWIST, SERPINB5, and SERPIN1 genes were studied for uterine leiomyoma etiopathogenesis.

Results: TWIST gene expression was significantly higher in the uterine leiomyoma tissue ($p<0.001$). SERPINB5 and SERPIN1 gene expression was decreased in the uterine leiomyoma tissue, but the differences were not statistically significant.

Conclusion: TWIST gene activity is significantly increased in leiomyoma tissue when compared to normal myometrium. In spite of the fact that the development of uterine leiomyomas is estrogen- and progesterone-dependent, myometrial cells could be triggered by the TWIST gene for uterine leiomyoma development. (J Turk Ger Gynecol Assoc 2014; 15: 92-5)

Key words: Uterine leiomyomas, etiopathogenesis, TWIST, SERPINB5, SERPIN1

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Introduction

Uterine leiomyomas (ULs) are typically defined as benign tumors of the myometrial smooth muscle tissue and found in up to 70% of women by the fifth decade of a woman's life (1, 2). UL is the most common type of solid tumor in women of reproductive age, with an incidence of 20-25% (3). UL is a monoclonal tumor of uterine smooth muscle cells and consists of large amounts of extracellular matrix that contains collagen, fibronectin, and proteoglycan (3). Although the majority of ULs is asymptomatic, up to 20% causes symptoms, like menorrhagia, chronic pelvic pain, pressure symptoms on the adjacent pelvic organ, and postpartum hemorrhage (3). Even though the pathogenesis of UL is not clearly known, there is considerable evidence that estrogens and progesterone play a role in growth (3, 4). Effective treatment strategies are limited by the narrow understanding of the pathogenesis of this disease. Cytogenetic analysis has demonstrated that ULs have multiple chromosomal abnormalities (5). Studies of UL specimens have shown that approximately 50% of these tumors have cytogenetic chromosomal alterations (5).

Twist homolog 1 (TWIST), also known as TWIST1, is a class II member of the basic helix-loop-helix transcription factor family (6). During embryonic development, TWIST plays an important role in specification of the mesoderm and differentiation of the mesoderm-derived tissues (6). Germline mutations in the coding sequence of the human TWIST gene have been observed in diverse types of cancers (7). TWIST also plays an important role in regulating smooth muscle cell differentiation and phenotypic modulation (8). The role of TWIST in the human uterus remains to be fully elucidated, but TWIST protein expression was observed in human myometrial smooth muscle cells (9). Serine peptidase inhibitor (SERPINB5), also known as maspin, is a member of the serpin family of serine protease inhibitors, and structurally, it is homologous to plasminogen activator inhibitor 1 (SERPIN1) (10). It is proposed that SERPINB5 is a tumor suppressor. However, the molecular role of SERPIN1 remains to be eluci-

Address for Correspondence: *Mehmet Sühha Bostancı, Department of Obstetrics and Gynecology, Sakarya University Faculty of Medicine, Sakarya, Turkey. Phone: +90 532 514 03 63 e.mail: msuhha@gmail.com*

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dated (10). SERPINB5 has recently been shown to bind directly to collagen, an interaction that may contribute to cell adhesion (11). SERPINB5 is shown to be differentially regulated in the progression of many types of solid tumors, like prostate, breast, and lung (12).

The aim of this study is investigate the role of the TWIST, SERPINB5, and SERPIN1 genes in uterine leiomyomas etiopathogenesis.

Material and Methods

Tissue Collection and Preparation

Twelve patients, aged between 39 and 58, who had a hysterectomy, were included in the study. Total abdominal hysterectomy was performed in 11 patients, and only 1 patient was operated on by vaginal hysterectomy technique. Tumor size was based on gross pathology after hysterectomy. The sizes of the leiomyomas were between 20 and 130 mm. Tissue samples were obtained from normal myometrium and leiomyoma (1 cm^3) tissues of the uterus of the patients and stored at -86°C. The present study complied with the ethical guidelines of the institutions involved, as it was approved by the Gazi University Ethical Committee, and informed consent was obtained from all subjects examined. Samples were divided two groups after histopathological evaluation of the uterus: control group (Group 1) as normal myometrial tissues and the study groups (Group 2) as leiomyoma tissue. TWIST, SERPINB5, and SERPIN1 were studied as 3 genes for uterine leiomyoma etiopathogenesis.

RNA Isolation

Tissue samples (1 cm^3) were homogenized in TRizol Reagent (TRizol Reagent; Invitrogen, New York, USA) using a homogenizer (T10 Basic Ultra-Turrax; IKA®-Werke GmbH & Co. KG, Staufen, Germany) according to the modified protocol published by Chomczynski (13, 14). Homogenized samples were subjected to isopropanol precipitation. RNA pellets were then purified using the Qiagen RNeasy mini-column kit (Qiagen RNeasy mini-column kit; Qiagen Sciences Inc. Valencia, Spain) according to the manufacturer's recommendation indicated in the kit procedures. RNA concentration was measured using a plate reader (Synergy HT Multi-Mode Microplate Reader; BioTek Synergy HT, Winooski, USA). The 260/280 ratios of all samples were above 1.9. RNA integrity was detected on an agarose gel (1%), and the ratios of 18S and 28S rRNA fragments were analyzed.

Real-time RT-PCR Arrays

One microgram total RNA per sample was converted into cDNA in a reverse transcriptase (RT) reaction. cDNA was synthesized from 1.5 μg of RNA sample using the SABiosciences RT² First Strand Kit. Gene expressions of myometrium and uterine leiomyoma tissue were assessed using a RT² Profiler™ PCR Array Human Cancer Pathway Finder (Cat. No. PAHS-033A) kit (RT² Profiler™ PCR Array Human Cancer Pathway Finder, PAHS-033A kit; SABiosciences Corporation, Frederick, MD, USA) with RT² Real-TimeTM SybrGreen PCR Master mix (RT² Real-TimeTM SybrGreen PCR Master mix, PA-012; SABiosciences Corporation, Frederick, MD, USA). Hypoxanthine phosphoribosyltransferase 1 (HPRT1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and β-actin (ACTB) genes were the housekeeping genes used for normalization. Results were calculated as fold-increase values of gene expression, as instructed by the kit supplier.

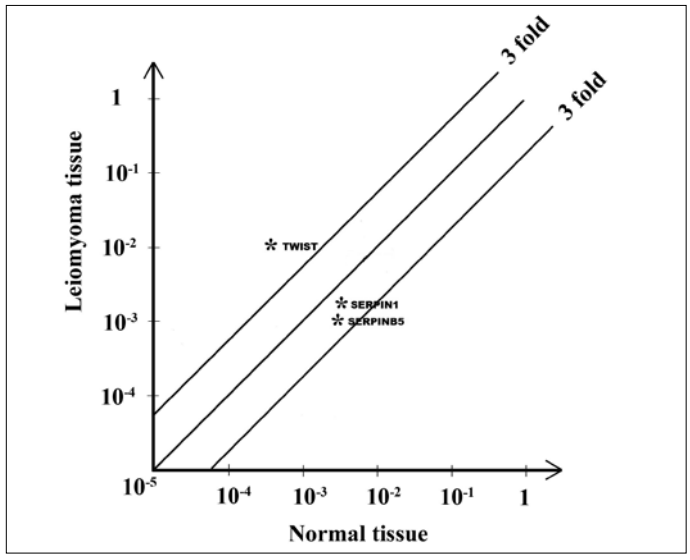

Figure 1. Result of polymerase chain reaction (PCR) array experiment, indicating the positions of several noteworthy genes based on their 3-fold differences in expression between normal myometrium and leiomyoma tissue (gene expression was calculated by the formula 2-ΔΔct using the ΔΔct method (threshold))

Statistical Analysis

Data obtained from densitometry were expressed as mean±SD. Relative changes as fold-change values in gene expression were calculated by the formula $2^{-\Delta\Delta ct}$ using the $\Delta\Delta c_t$ method (threshold) (Statistical Package for Social Sciences for Windows, version 7.5; SPSS, Inc., Chicago, IL, USA). Statistical analysis was conducted using the Mann-Whitney U-Test. Statistical significance was established at p values of less than 0.05.

Results

We examined the gene expression profiles exhibited by leiomyoma and normal myometrium tissue. Mean age of the patients was 46.58±4.75 years. Mean value of gravidity for patients was 3.17±1.80. For this study, TWIST, SERPINB5, and SERPIN1 were studied as 3 genes. Figure 1 displays the results from the PCR array experiment, indicating the positions of several noteworthy genes based on their 3-fold differences in expression between normal myometrium and leiomyoma tissue by the formula $2^{-\Delta\Delta ct}$ using the $\Delta\Delta c_t$ method (threshold). The TWIST gene demonstrated at least a 3-fold difference, but the others did not. Upregulation was observed for the TWIST gene in the leiomyoma sample when compared to normal uterine myometrium. The SERPINB5 and SERPIN1 genes appeared to be downregulated in the leiomyoma sample. The results for all genes are presented in Table 1. According to this, in the TWIST gene, statistical significance was found between groups

Table 1. The expression of TWIST, SERPINB5, and SERPIN1 genes in uterine leiomyoma and normal myometrium tissue

Genes	Gene Bank	Function	p value	Fold-change (Group 2/Group 1)
TWIST	NM_000474	Twist homolog 1 (Drosophila)	0.001	4.84
SERPINB5	NM_002639	Serpin peptidase inhibitor, clade B (ovalbumin), member 5	0.136	-1.33
SERPIN1	NM_000602	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	0.197	-1.19
TWIST: twist homolog 1; SERPINB5: serine peptidase inhibitor; SERPIN1: plasminogen activator inhibitor 1				

($p<0.001$). The downregulation was not statistically significant for the SERPINB5 and SERPIN1 genes ($p>0.05$).

Discussion

Uterine leiomyomas are very common in women, but knowledge of the tumor biology is very limited. Clinical and molecular studies have demonstrated that ULs are not a single entity but a clinically and genetically heterogeneous disease (2). ULs are composed of large amounts of extracellular matrix containing collagen, fibronectin, and proteoglycan (15). There is increasing evidence that multiple genomic adjustments may play a role in the initial growth of leiomyoma and that molecular and cellular mechanisms, rather than hormonal factors alone, play a major role in their subsequent growth. TWIST has a role in mesoderm-associated organogenesis; the exact molecular mechanisms by which TWIST controls mesenchymal tissue formation remain largely undefined (16). TWIST is postulated to perform its central regulatory roles in organogenesis at least partially via its control over basic fibroblast growth factor (βFGF) and transforming growth factor beta (TGF-β) signaling (17). In addition to its essential role in modulating mesenchymal tissues critical for organogenesis, TWIST is also expressed in and associated with many types of tumors, including breast cancer, hepatocellular carcinoma (HCC), prostate cancer, and gastric cancer (7, 18-20). TWIST upregulates vascular endothelial growth factor (VEGF) and N-cadherin expression in HCC, and this condition suggests that TWIST may also play an important role in HCC angiogenesis (19). TWIST also enhances VEGF production in prostate cancers (20). VEGF is an angiogenic factor; its enhanced production may accelerate angiogenesis associated with the metastasis of these tumors (19, 20). βFGF and its receptors are expressed in normal myometrium and leiomyoma (21). Expression of βFGF and its receptors in leiomyoma is greater than in myometrium (21). TWIST also enhances βFGF expression (22). In our study, we found that TWIST gene expression was increased approximately 5-fold in leiomyoma uterine tissue when we compared with normal uterine myometrial tissue. Based on the information in the literature, TWIST may play role in the formation of ULs by enhancing angiogenesis and growth. SERPINB5 is expressed in many tissues, including prostate, mammary gland, skin, stomach, and uterus (7, 11, 23). SERPINB5 and SERPIN1 have been identified as potent angiogenesis inhibitors. However, the molecular mechanism responsible for their anti-angiogenic property is unclear. The loss of SERPINB5 expression is significantly correlated with increased expression of VEGF in tumor cells (23). As a tumor suppressor, SERPINB5 functions to inhibit tumor cell migration and invasion and induces tumor cell apoptosis (23). In our study, SERPINB5 and SERPIN1 expression in leiomyoma tissue was decreased when compared to normal uterine myometrium. But, the reduction was not statistically significant. More comprehensive studies with larger numbers of cases may be useful to have a more accurate decision. The limited number of cases in our study can be an issue. For this reason, it is difficult to comment on the role of these two genes in the formation of uterine leiomyoma.

The etiopathogenesis of UL is not fully explained. ULs are not a single entity but a clinically and genetically heterogeneous disease. We showed TWIST gene upregulation for UL tissue when compared to normal myometrial tissue. In spite of the fact that the development of uterine leiomyomas is estrogen- and progesterone-dependent, myometrial cells could be triggered by the TWIST gene for UL development. This situation may be considered a promising sign in preventing the development UL. More comprehensive studies can be done on the role of the TWIST gene and treatment modalities for UL through this gene.

Ethics Committee Approval: *Ethics committee approval was received for this study from Gazi University Scientific and Ethical Committee.*

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