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Gonadotropin-releasing hormone agonist induces downregulation of tensin 1 in women with endometriosis

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Abstract

Introduction: Many cell migration-related molecules are associated with endometriosis. Tensin 1 (TNS1), which has been implicated in cell migration, may play a role in endometriosis. The study goal was to evaluate the TNS1 expression in endometrial tissue and serum from women with endometriosis treated with gonadotropin-releasing hormone agonist (GnRHa).

Material and methods: Tissue and serum samples were collected from women with endometriosis who were treated (n = 29) with GnRHa or untreated (n = 30). *TNS1* mRNA was examined using quantitative PCR. TNS1 protein levels in tissue and serum samples were investigated using Western blot, immunohistochemistry and ELISA. Eleven women with endometriosis participated in a follow-up investigation of serum TNS1 before and after GnRHa treatment.

Results: TNS1 mRNA ($P = 0.006$) and protein ($P = 0.001$) were significantly downregulated in endometriotic tissue from women with endometriosis who received GnRHa. Immunolocalization of TNS1 showed strong expression in the epithelial and stromal cells of endometriotic tissue from women untreated with GnRHa, whereas endometriotic tissue from GnRHa-treated women showed low TNS1 expression. Follow-up monitoring of serum TNS1 concentration in 11 women showed an average decrease in concentration of 53%, from 294.9 ± 66.69 to 140.3 ± 55.21 pg/mL, following GnRHa treatment ($P = 0.003$).

Conclusions: GnRHa induces downregulation of TNS1 in tissue and serum in women with endometriosis. These results emphasize the importance TNS1 as a potential therapeutic molecular target for the treatment of endometriosis with GnRHa.

KEYWORDS
endometriosis, gonadotropin-releasing hormone agonist, tensin 1

Abbreviations: ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; GnRHa, gonadotropin-releasing hormone agonist; qPCR, quantitative PCR; TNS1, tensin 1.

Yang and Tzeng contributed equally to this manuscript.

1 | INTRODUCTION

Endometriosis is a benign gynecological disorder characterized by the presence and proliferation of extrauterine endometrial tissue[1] and is a risk factor for infertility.[2] Retrograde menstruation is considered to be the primary etiology, followed by the processes of attachment, invasion, proliferation and maintenance.[3] Although the underlying pathogenesis of endometriosis is still poorly understood, many cell migration-related and invasion-related molecules are associated with the progression of endometriosis.[4,5]

Tensin 1 (TNS1) is a focal adhesion phosphoprotein molecule localized in cytoplasm that plays a role in the transmembrane junctions between the extracellular matrix and cytoskeleton.[6] Cells lacking TNS1 migrate more slowly than their normal counterparts, demonstrating that TNS1 is critical for normal cell migratory processes.[7] TNS1 also plays a role in cell survival,[8] apoptosis,[9] angiogenesis,[10] inflammation[11,12] and hypoxia.[13,14]

Expression of the tensin family of proteins occurs in many organs, including the heart, skeletal muscles, kidneys, lungs, small intestine, liver, colon, prostate, testis, and ovaries.[15] Very low or no expression has been found in the brain, thymus, and circulating leukocytes.[15] In the gynecologic system, TNS1 is involved in implantation[16] and oogenesis,[17] and is expressed in the endometrium.[18] A phylogenetic study of ovarian endometriosis revealed that TNS1 is overexpressed in endometriotic tissue compared with eutopic tissues.[19] However, no reports to date have described the effect of hormonal treatment on TNS1 expression in women with endometriosis.

A woman's hormonal status has long been considered to be essential in the development and maintenance of endometriosis. One of the most widely used agents for the medical treatment of endometriosis is gonadotropin-releasing-hormone agonist (GnRHa). A great deal of evidence supports the efficacy of GnRHa for pain control and reducing the size of endometriotic lesions.[20,21]

Thus, we sought to evaluate the effect of GnRHa on TNS1 expression in tissues and serum among women with endometriosis.

Key message

TNS1 expression in tissue and serum is downregulated following GnRHa treatment in women with endometriosis. TNS1 expression is independent of menstrual cycle and independent of GnRHa treatment duration.

We hypothesized that TNS1 would be downregulated in women with endometriosis who receive GnRHa treatment compared with women who are not treated with GnRHa. To our knowledge, this is the first study to investigate TNS1 expression in women with endometriosis following GnRHa treatment.

2 | MATERIAL AND METHODS

2.1 | Specimen collection

We recruited women scheduled for initial laparoscopic surgery for ovarian endometriosis during 2011-2016. Participants were confirmed based on histology and all had moderate-to-severe endometriosis based on the classification of the American Society for Reproductive Medicine.[22] The participants' profiles are shown in Table 1.

Sample collection took place in two phases. A pilot study was conducted to measure *TNS1* mRNA level using 26 endometriotic tissue samples from women with endometriosis who were (n = 12) or were not (n = 14) receiving GnRHa treatment. In the next phase, we further confirmed the pilot protein level results with a second cohort of 33 women with endometriosis who were (n = 17) or were not (n = 16) receiving GnRHa treatment. Among these 33 women, 27 contributed both tissue and serum samples, three contributed only a tissue sample, and three contributed only a serum sample. Normal endometrial samples were obtained from 42-year-old (at proliferative phase) and 43-year-old (at secretory phase) women

TABLE 1 Baseline characteristics of study population

	Women with endometriosis	Women with endometriosis	
	$GnRHa^-$	$GnRHa^+$	*P* value
Total, n	30	29	
Age, y	32.37 ± 5.87 (22-44)	34.17 ± 3.77 (25-43)	0.167
BMI, kg/m^2	21.6 ± 4.17 (16-35)	21.38 ± 2.95 (17-29)	0.815
Dysmenorrhea, %	22 (73.33)	25 (86.21)	0.219
Smoking, %	6 (20)	11 (37.93)	0.128
Alcohol, %	2 (6.67)	4 (13.79)	0.365
Menstrual cycle		Not evaluated	
Proliferative phase, %	14 (46.67)		
Secretory phase, %	16 (53.33)		

Mean ± SD (range) is indicated for continuous variables.
BMI, body mass index; GnRHa, gonadotrophin-releasing hormone agonist; n, number of patients.

without endometriosis. These samples served as controls for the immunohistochemistry experiment. Eutopic endometrium samples from women with endometriosis were also obtained from GnRHa treated and untreated women. In addition, a follow-up study of 11 women was conducted to compare serum TNS1 level before and after GnRHa treatment (Table 2). Women signed an informed consent form twice, first at recruitment in the outpatient department when the blood sample was collected (before GnRHa treatment) and again before receiving laparoscopic surgery (after GnRHa treatment) for inclusion in the follow-up study. In the untreated group, we analyzed TNS1 expression based on menstrual phase, which was assigned using the last menstrual period method. In the treatment group, TNS1 expression analysis was based on GnRHa treatment duration. We excluded women with endometriosis who had a history of hormonal treatments other than GnRHa (eg Danazol, follicle-stimulating hormone or other hormone-based drugs). The GnRHa treatment dose was 1.875 mg/injection (half dose) per month. Most women in this study received 1 month of GnRHa treatment; however, for some, another shot of GnRHa was given to maintain treatment effectiveness if they were unable to schedule surgery 1 month after GnRHa treatment.

2.2 | RNA isolation and qPCR analysis

Tissue samples used for real-time quantitative PCR (qPCR) were obtained from ectopic sites during laparoscopic surgery, snap frozen in liquid nitrogen, and kept at −80°C until they were used for the experiment. *TNS1* gene expression in endometriotic tissues treated with and without GnRHa was evaluated by qPCR using SYBR Premix Ex Taq (Applied Biosystems Inc., Foster City, CA, USA). Total RNA was extracted using the TRIzol (Invitrogen, Carlsbad, CA, USA) method. Complementary DNA (cDNA) was generated, according to the manufacturer's instructions, from total RNA using a reverse transcription kit (Invitrogen). The relative level of *TNS1* gene expression was compared with an internal control, TATA binding protein (*TBP*). Oligonucleotide primers for *TNS1* (forward: 5′ GACGCGGGCTAAAGTGAAGT 3′; reverse: 5′ GTGACTGTCGCGGATGATGA 3′) and TBP (forward: 5′ TGCACAGGAGCCAAGAGTGAA 3′; reverse: 5′ CACATCACAGCTCCCCACCA 3′) were designed using primer EXPRESS v2.0 Software (Applied Biosystems Inc.). Experiments were performed in duplicate.

2.3 | Western blot analysis

Western blot analysis was performed to determine TNS1, estrogen receptor alpha (ERα), and estrogen receptor beta (ERβ) protein levels in endometriotic tissues. Briefly, endometriotic tissues from the treated and untreated groups were homogenized in mammalian tissue lysis buffer (CelLytic MT; Sigma-Aldrich, St. Louis, MO, USA) with protease inhibitor cocktail (Sigma-Aldrich). The total protein content was measured using a Bio-Rad DC protein assay kit (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada). Equal amounts of total protein (30 μg) from each sample were loaded onto a 10% SDS–polyacrylamide gel for electrophoresis, and proteins were transferred to a polyvinylidene fluoride membrane (GE Healthcare, Amersham, UK; 0.45 μm) using a Semi-Dry Electrophoretic Transfer Cell (Bio-Rad Laboratories Ltd.) for 1 hour. Membranes were incubated in 5% nonfat milk for 1 hour and then incubated overnight at 4°C with mouse anti-TNS1 (ab167660; Abcam, Cambridge, UK), mouse anti-ERα (sc-56833; Santa Cruz Biotechnology, Dallas, TX, USA), and rabbit anti-ERβ (ab3576; Abcam) antibodies. Subsequently, to demonstrate that equal amounts of protein were loaded among the samples, the membranes were stripped and incubated with mouse anti-glyceraldehyde 3-phosphate dehydrogenase (ab8245; Abcam) antibody as the loading control. The membranes were then probed with the appropriate secondary antibody labeled with horseradish peroxidase (GE Healthcare). Signals were visualized using enhanced

TABLE 2 Characteristics of women with endometriosis involved in the follow-up study

Patients	Age (y)	BMI (kg/m^2)	Duration of GnRHa tx (mo)	Diagnosis at laparoscopy
Patient 1	32	19.88	1	Stage IV endometriosis
Patient 2	33	22.04	2	Stage III endometriosis
Patient 3	32	20.57	2	Stage III endometriosis
Patient 4	38	18.61	1	Stage III endometriosis
Patient 5	40	20.83	1	Stage III endometriosis
Patient 6	35	19.03	1	Stage IV endometriosis
Patient 7	33	20.78	1	Stage IV endometriosis
Patient 8	33	22.59	2	Stage III endometriosis
Patient 9	33	18.56	2	Stage III endometriosis
Patient 10	35	17.27	2	Stage IV endometriosis
Patient 11	33	21.74	1	Stage IV endometriosis

Serum samples from women with endometriosis were collected before and after GnRHa and analyzed using ELISA.
BMI, body mass index; GnRHa, gonadotropin-releasing hormone agonist; tx, treatment.

chemiluminescence (GE Healthcare) and quantitated with a BioSpectrum AC Imaging System (UVP, Upland, CA, USA).

2.4 | Immunohistochemistry

Immunohistochemistry was performed to localize protein expressions of TNS1, ERα and ERβ in endometriotic tissues and in eutopic endometrium from women with endometriosis in treated and untreated groups. Tissue samples were collected during laparoscopy resection, immediately fixed in 10% paraformaldehyde for 12 hours, and embedded in paraffin. Paraffin blocks for each sample were cut at 3 μm thickness. Eutopic endometrial tissues from women without endometriosis were used as a control, and routine hematoxylin and eosin staining was performed to provide a tissue overview. Immunostaining was executed using a Novolink Polymer Detection System kit (RE7140-K; Leica Biosystems, Newcastle upon Tyne, UK) following the manufacturer's instructions. Antigen retrieval was performed in Novocastra Epitope Retrieval Solution pH 9.0 (Leica Biosystems) for TNS1, and pH 6.0 (Leica Biosystems) for ERα and ERβ. The sections were incubated with goat anti-TNS1 (OAEB00321; Aviva Systems Biology, San Diego, CA, USA), rabbit anti-ERα (ab39642; Abcam) and rabbit anti-ERβ (ab3576; Abcam) overnight at 4°C followed by the secondary antibody for 30 minutes at room temperature. 3,3′-Diaminobenzidine chromogen was applied in its working solution and counterstained with hematoxylin. Negative controls were performed using the same protocol, and the primary antibody was replaced with goat immunoglobulin G (IgG) isotype control antibody (LS-C351732; LifeSpan Biosciences Inc., Seattle, WA, USA) or rabbit IgG isotype control antibody (ab199376; Abcam). Representative images were captured using SPOT imaging software.

2.5 | Enzyme-linked immunosorbent assay

The blood samples from women with endometriosis were collected by peripheral venipuncture before anesthesia was administered. Serum was extracted by centrifugation at 1670 ***g*** for 10 minutes and stored at −80°C until used for ELISA. Serum levels of TNS1 were quantified using an ELISA kit (MyBioSource, San Diego, CA, USA; MBS905546) and performed according to their protocol. This assay uses anti-TNS1 capture antibody coated on a 96-well plate. Standards and samples were pipetted into the wells, and the target protein present in a sample was bound to the wells by the immobilized antibody. Unbound proteins were removed by extensive washing, and a biotinylated anti-TNS1 antibody was added. After washing, avidin-conjugated horseradish peroxidase substrate was added to the wells for color development. Level of bound TNS1 protein, in proportion to the intensity of developed color, was measured using a microplate reader at 450 nm. A standard curve was plotted for each plate, and the serum level of TNS1 in each sample was derived by interpolation using the standard curve. Each sample was analyzed in duplicate, and the mean value was calculated. Estradiol serum level was measured by chemiluminescence immunoassay (Union Clinical Laboratory, Taipei, Taiwan).

2.6 | Statistical analyses

Data were analyzed using GraphPad PRISM version 7 for Mac (GraphPad Software, San Diego, CA, USA). Participant demographic and anthropomorphic characteristics were compared between the treated and untreated groups using unpaired samples and two-tailed *t* tests for continuous variables (such as age and body mass index); discrete variables (such as dysmenorrhea, smoking and alcohol) were analyzed using Chi-square. The Mann–Whitney *U* test was used to compare TNS1 expression between the groups. To evaluate the concentration of TNS1 in serum in women with endometriosis before and after GnRHa treatment, we used Wilcoxon signed rank test. Women in the follow-up group were also included in the untreated group before they received GnRHa treatment, including analyses based on menstrual cycle; their follow-up TNS1 concentrations posttreatment were also included in the treated group analyses, including duration of treatment analyses. Differences were considered to be statistically significant when $P \leq 0.05$.

2.7 | Ethical approval

The study was approved by the Joint International Review Board of Taipei Medical University Hospital, Taipei, Taiwan (TMU-JIRB 201006002 and TMU-JIRB 201305035). All participants provided written informed consent.

3 | RESULTS

3.1 | Study sample characteristics

The baseline characteristics of the participants in the two groups are summarized in Table 1. There were no significant between-group differences in age, body mass index, dysmenorrhea, smoking or alcohol use ($P > 0.05$). Characteristics of the participants in the follow-up study, in which we assessed TNS1 serum level before and after GnRHa treatment, are described in Table 2.

3.2 | Expression of TNS1 mRNA in endometriotic tissue

TNS1 mRNA expression was quantified in endometriotic tissue from women with endometriosis who were treated (n = 12) or not treated (n = 14) with GnRHa (Figure 1A). *TNS1* mRNA expression in endometriotic tissue from the treated group was significantly lower than from the untreated group ($P = 0.006$). Further analysis of the untreated group showed that there was no significant difference between proliferative and secretory phases ($P = 0.8$) (Figure 1B). In the treated group, *TNS1* mRNA did not differ significantly based on length of GnRHa administration (1 month vs ≥2 months) ($P = 0.48$) (Figure 1C).

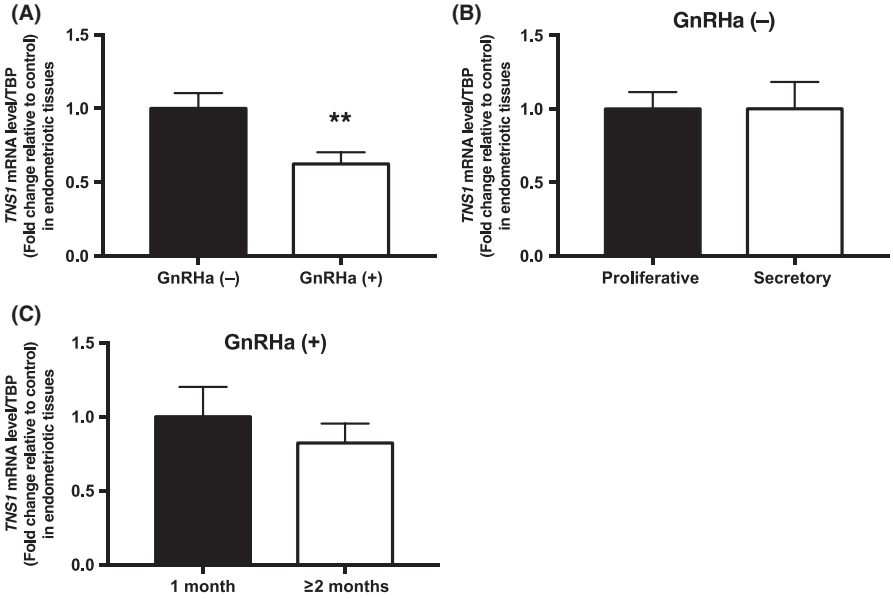


FIGURE 1 Expression of *TNS1* mRNA in endometriotic tissues analyzed by quantitative real-time PCR. (A) Expression of *TNS1* mRNA in endometriotic tissues from women treated ($GnRHa^+$, n = 12) and not treated ($GnRHa^-$, n = 14) with gonadotrophin-releasing hormone agonist; $^{**}P = 0.006$. (B) *TNS1* mRNA level for the $GnRHa^-$ group in the proliferative phase (n = 7) and secretory phase (n = 7) of the menstrual cycle; $P = 0.8$. (C) *TNS1* mRNA expression in the $GnRHa^+$ group between women with endometriosis treated for 1 month (n = 3) or ≥2 months (n = 9); $P = 0.48$. The TATA box binding protein (*TBP*) gene was used as an internal control. The qPCR experiment was carried out in duplicate; the bar chart shows the deviation between patients, and data are expressed as the mean ± SEM; n = number of patients. The qPCR data for *TNS1* mRNA were expressed relative to *TBP* mRNA and normalized to the untreated group. GnRHa, gonadotropin-releasing hormone agonist; TNS1, tensin 1

3.3 | TNS1 protein in endometriotic tissue

Expression of *TNS1* mRNA was confirmed based on protein levels. The protein level from *TNS1* mRNA was consistent with the mRNA level. Figure 2A shows that treatment with GnRHa induced down-regulation of TNS1 protein compared with the control ($P = 0.001$). In addition, TNS1 protein was consistent between samples taken during the proliferative and secretory phases ($P = 0.6$) (Figure 2B) in the untreated group. In the treated group, duration of GnRHa treatment of 1 month or ≥2 months did not affect the TNS1 protein level ($P = 0.17$) (Figure 2C). Furthermore, we did not find any significant differences in ERα or ERβ in protein levels in endometriotic tissues from women treated or not treated with GnRHa (Supporting Information Figure S2).

3.4 | Localization of TNS1

Immunohistochemistry was used to evaluate the cellular localization of TNS1 protein in normal endometrium, eutopic endometrium from women with endometriosis, and endometriotic tissues (Figure 3). In normal endometrium from women without endometriosis, samples taken during both proliferative and secretory phases demonstrated mostly negative epithelial and stromal TNS1 immunostaining; only light stromal staining could be seen. In contrast, eutopic endometrium from women with endometriosis treated (Figure 3F) and not treated with GnRHa during proliferative (Figure 3D) and secretory phases (Figure 3E) showed high expression of TNS1 compared with normal endometrium. Eutopic endometrium from women with endometriosis treated with GnRHa showed that TNS1 staining was lighter compared with the eutopic endometrium from untreated women (Figure 3D,E). Furthermore, endometriotic cyst samples from women untreated with GnRHa showed diffuse TNS1 immunostaining in the stroma cell (Figure 3N-P) and positive staining in the cytoplasm of the epithelial cell (Figure 3L,M). Variation in TNS1 immunostaining in the stromal cells of untreated endometriotic cysts was observed, whereas stromal cells adjacent to the epithelial cells (Figure 3L,M) showed lower expression compared with other samples (Figure 3N-P). Endometriotic cysts from women treated with GnRHa showed diminished immunostaining in epithelial (Figure 3Q) and stromal cells (Figure 3R-U). Investigations of ERα and ERβ expressions in eutopic endometrium of women with endometriosis revealed less expression of ERα (Figure S3N) but not ERβ in treated women compared with untreated women (Figure S3H,K).

3.5 | TNS1 serum concentrations

Tensin 1 serum was measured in lower concentrations in the GnRHa-treated group (n = 25) compared with the untreated group (n = 27), but this difference was not statistically significant (190.6 ± 28.75 pg/mL vs 243.8 ± 30.02 pg/mL, respectively; $P = 0.09$) (Figure 4A). In the untreated group, we further analyzed TNS1 serum level based on menstrual phase and found no significant difference between the proliferation and secretory phases (251.7 ± 53.78 pg/mL vs 235.3 ± 25.86 pg/mL, respectively; $P = 0.55$) (Figure 4B). Duration

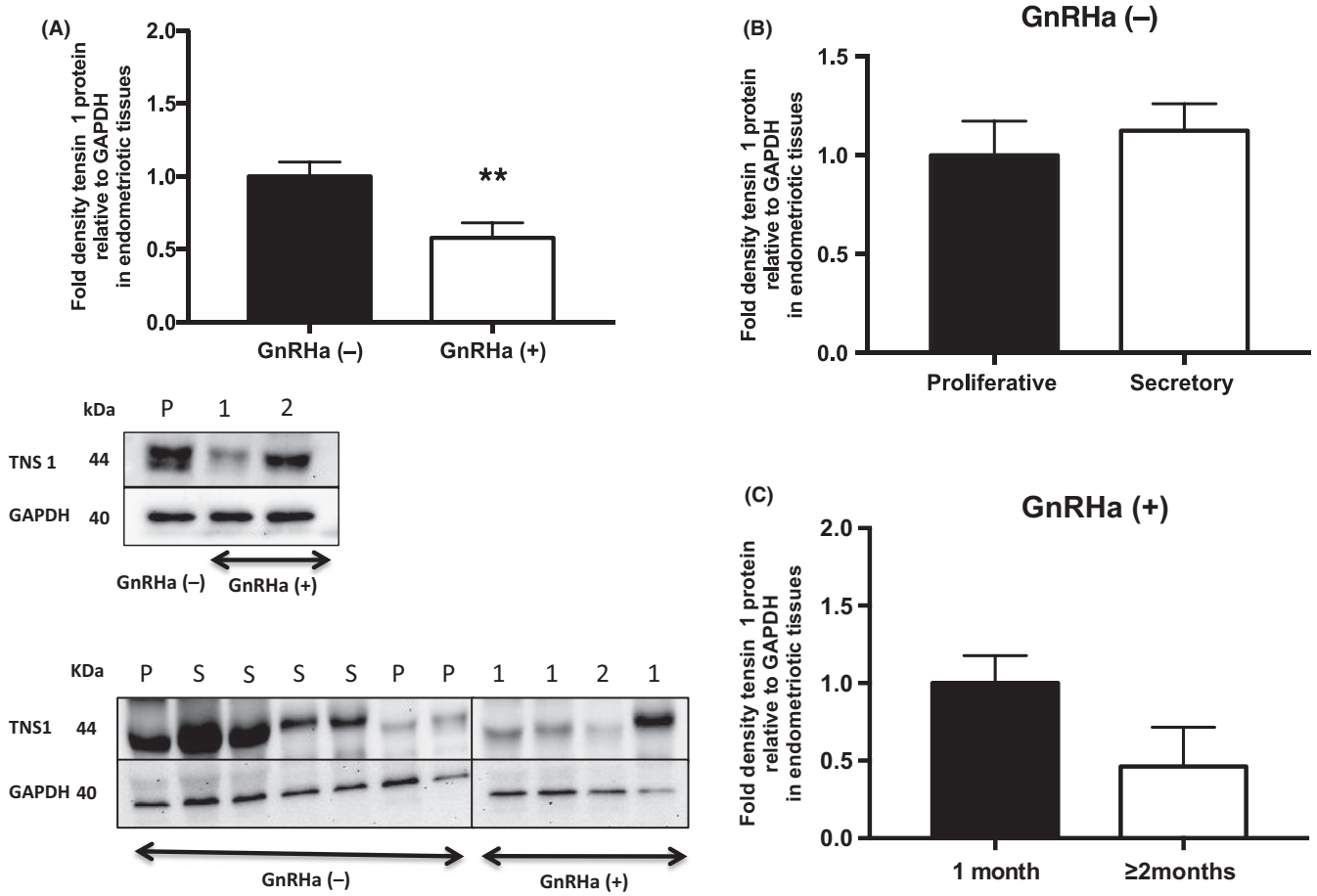


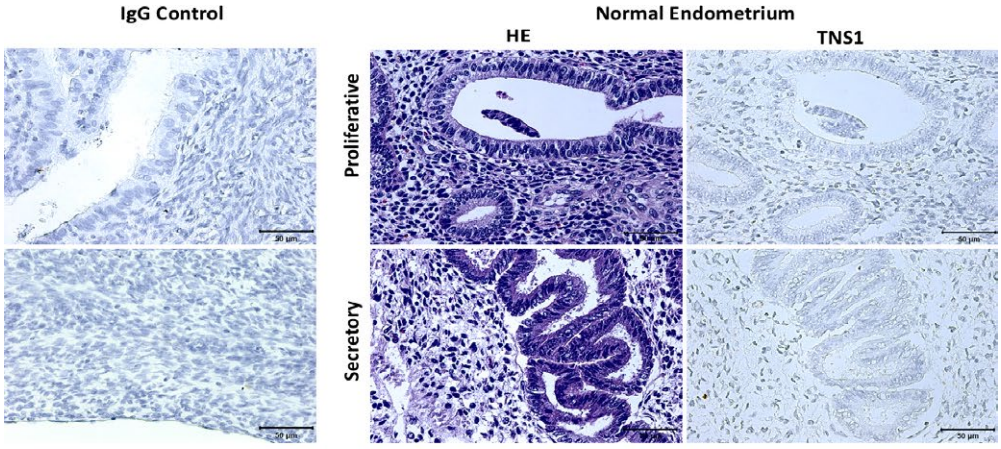
FIGURE 2 TNS1 protein level in endometriotic tissues analyzed with Western blotting. (A) TNS1 protein level in women with endometriosis treated with gonadotrophin-releasing hormone agonist ($GnRHa^+$, n = 14) and in those not treated with GnRHa ($GnRHa^-$, n = 16); $^{**}P = 0.001$. Representative blots are shown below the graph; S = secretory, P = proliferative, 1 = patients treated for 1 month, and 2 = patients treated for 2 months. (B) TNS1 protein level from the $GnRHa^-$ group in the proliferative (n = 7) and secretory (n = 9) phases; $P = 0.6$. (C) TNS1 protein level in the $GnRHa^+$ group, between women with endometriosis treated for 1 month (n = 11) and ≥2 months (n = 3); $P = 0.17$. The data are expressed as the mean ± SEM; n = number of patients. GnRHa, gonadotropin-releasing hormone agonist; TNS1, tensin 1

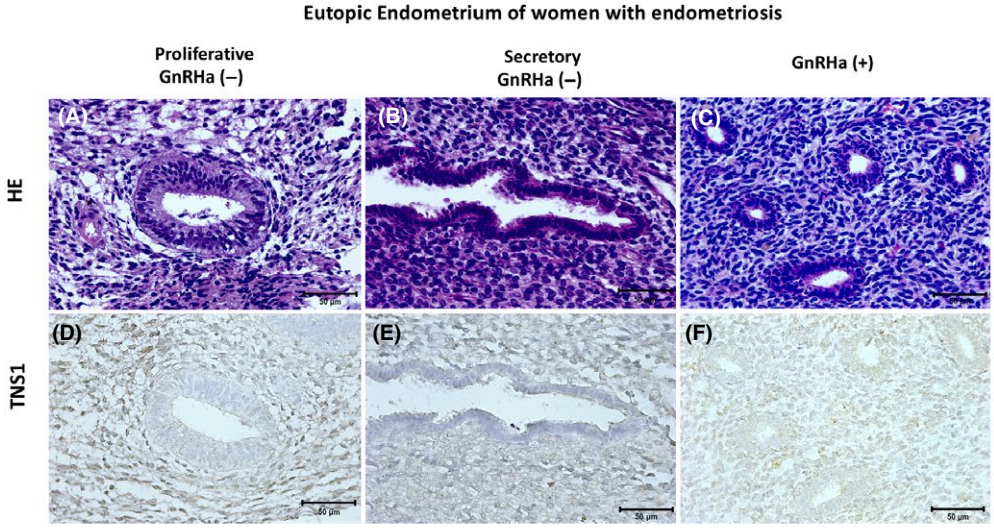
of GnRHa treatment had no effect on TNS1 serum level between women treated for 1 month (211.4 ± 34.69 pg/mL) compared with ≥2 months (153.5 ± 51.03 pg/mL) ($P = 0.36$) (Figure 4C). Follow-up monitoring of TNS1 serum concentration after GnRHa treatment in 11 women showed a 53% decrease in the average concentration from pre- to posttreatment (294.9 ± 66.69 pg/mL vs 140.3 ± 55.21 pg/mL, respectively; $P = 0.003$). Estradiol levels among women with endometriosis were significantly lower in the treated (17.91 ± 4.074 pg/mL) than in the untreated group (107.7 ± 27.14 pg/mL) ($P = 0.0002$) (Supporting Information Figure S1A). In the follow-up study, serum estradiol showed a significantly lower concentration (83%) from pre- to posttreatment (75.87 ± 22.33 pg/mL vs 13.11 ± 1.156 pg/mL, respectively; $P = 0.0156$) (Figure S1B). Although serum estradiol was lower in women treated for ≥2 months (15.78 ± 2.132 pg/mL) compared with 1 month (19.43 ± 7.006 pg/mL), this difference was not statistically significant ($P = 0.58$) (Figure S1C).

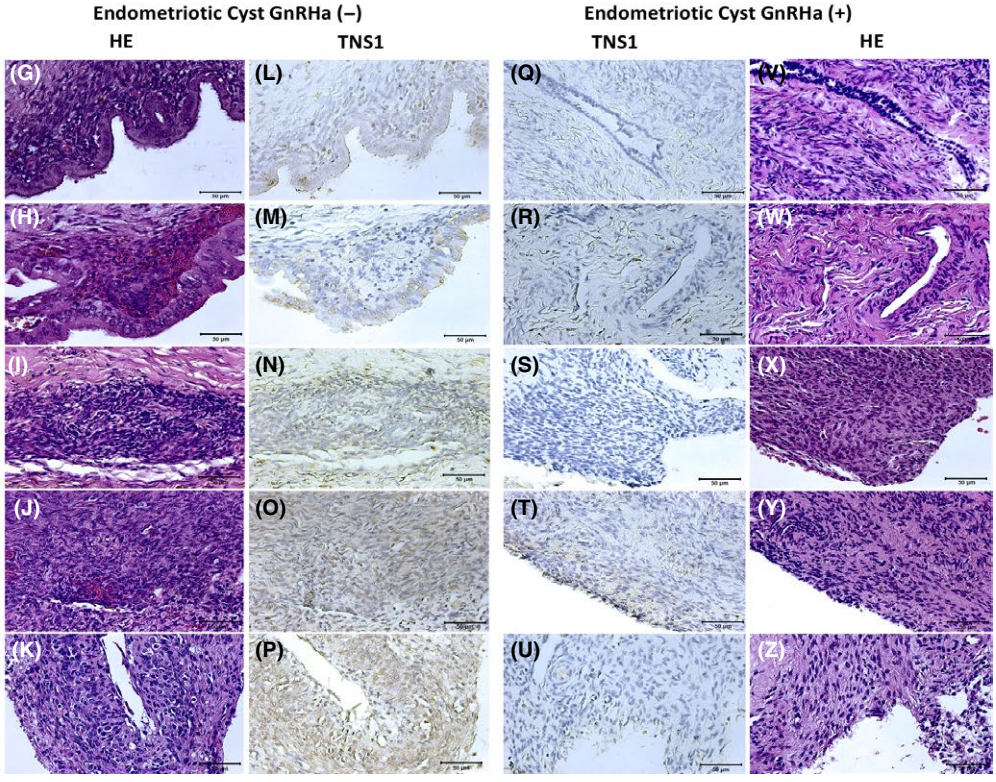
4 | DISCUSSION

This study shows that TNS1 mRNA and protein expression are downregulated in women with endometriosis following GnRHa treatment.

FIGURE 3 Immunostaining of TNS1 in endometrium and endometriotic cyst treated with and without GnRHa. Expression of TNS1 in eutopic endometrium of women with endometriosis is shown in (D) (proliferative phase from untreated woman), (E) (secretory phase from untreated woman) and (F) (treated woman). Immunostaining of TNS1 in endometriotic cyst from five patients in each group; women with endometriosis who were treated (Q-U) and untreated (L-P) with GnRHa are shown. Nonimmune IgG stain was used as negative control. Hematoxylin and eosin (HE) stain was used as tissue overview (A-C, G-K, & V-Z). TNS1 immunostaining is indicated in brown and counterstaining with hematoxylin is blue. Original magnification 400×. Scale bar: 50 μm. GnRHa, gonadotropin-releasing hormone agonist; TNS1, tensin 1







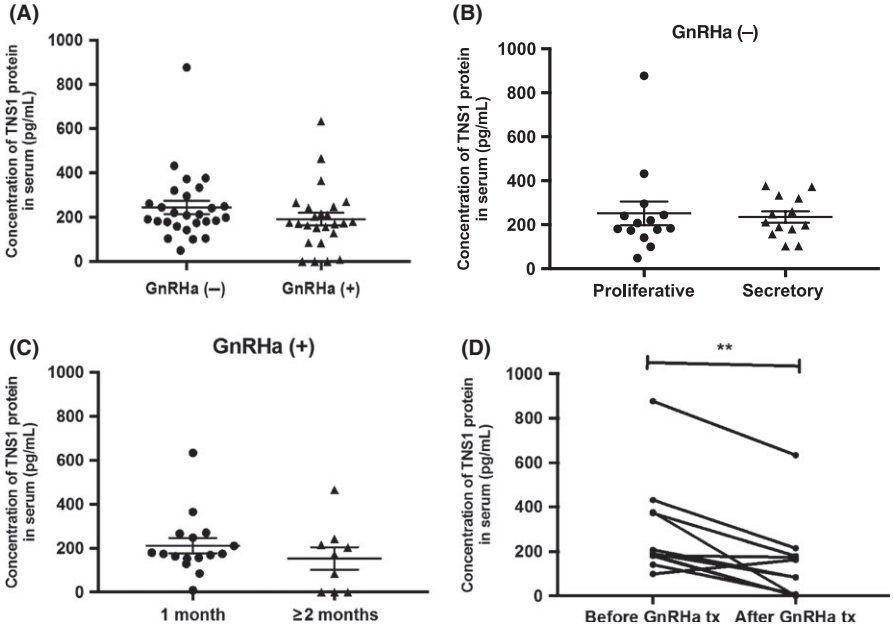


FIGURE 4 Serum level of TNS1 in women with endometriosis measured by ELISA. (A) Serum TNS1 concentration in women treated with gonadotrophin-releasing hormone agonist ($GnRHa^+$, n = 25) and those not treated with GnRHa ($GnRHa^-$, n = 27); $P = 0.09$. (B) Serum TNS1 concentration in the $GnRHa^-$ group during the proliferative (n = 14) and secretory (n = 13) phases of the menstrual cycle; $P = 0.55$. C, Serum TNS1 concentration in the $GnRHa^+$ group, for women treated for 1 month (n = 16) or ≥2 months (n = 9); $P = 0.36$. The data are expressed as the mean ± SEM. (D) TNS1 concentration was measured in serum from 11 women with endometriosis before and after GnRHa treatment, $^{**}P = 0.003$; n = number of patients; tx = treatment

Endometriosis is a relatively common, benign gynecological disorder characterized by many malignant cell features, including migration and invasion. Here we demonstrated that a migration-related protein, TNS1, is expressed in tissues and serum of women with endometriosis, suggesting that TNS1 may be involved in the pathogenesis of endometriosis. The function of TNS1 related to migration has been shown previously.[7,23]

Our current experiments have demonstrated that GnRHa induces the downregulation of TNS1 mRNA and protein level in tissues and serum of women with endometriosis. The study also indicates that the biological effect of GnRHa on TNS1 is not only systemic but also local to endometriotic tissues. GnRHa treatment is well known to induce hypoestrogenemia in women with endometriosis. Previous studies have demonstrated the effects of estrogen on TNS1 expression; TNS1 is upregulated by estradiol,[24] and treatment with a high affinity estrogen receptor antagonist in the murine uterus downregulates its expression.[25] Our results reveal that women with endometriosis who received GnRHa treatment have lower estradiol levels compared with those who are untreated (Figure S1). We have found that in eutopic endometrium from women with endometriosis, treatment with GnRHa induces reduced expression of ERα, but not in ERβ (Supporting Information Figure S3). In contrast, ERα and ERβ expressions in endometriotic tissues remained unchanged following GnRHa treatment (Figure S2). This finding is consistent with a previous study showing that treatment with leuprolide acetate has no effect on ERα and ERβ expression in endometrioma.[26] With regard to broader implications, our data provide new evidence that hypoestrogenemia during GnRHa treatment may reduce the ability to express TNS1 in women with endometriosis and may thus lead to suppression of its migration. Further investigation is needed to determine the role of TNS1 in the pathogenesis of endometriosis and its relation to estrogen receptors.

We found that the serum concentration of TNS1 in GnRHa-treated women with endometriosis, compared with those untreated with GnRHa, was lower (Figure 4A), although this difference was not statistically significant. However, in the follow-up study, serum TNS1 concentration declined significantly after GnRHa treatment (Figure 4D), indicating that knowing a patient's baseline TNS1 serum level may be very important for predicting how effectively GnRHa administration will reduce her serum TNS1. Ten of 11 women in the follow-up study showed a decrease in TNS1 after GnRHa treatment. The duration of GnRHa treatment in these 11 women ranged from 1 to 2 months (Table 2). Monitoring the within-patient serum level of this protein before and after treatment may allow improved evaluation of response to treatment compared with measuring TNS1 serum between treated and untreated groups as we did in this study. Accordingly, we plan to follow the serum TNS1 response to GnRHa treatment in a longitudinal cohort study with a larger sample size.

Because endometriosis is a hormone-dependent disorder, we assessed whether the menstrual cycle affected TNS1 expression. The endometrium undergoes important structural modifications, combined with changes in the specific functions of its cells, in response to the cyclic variations in estrogen and progesterone during the menstrual cycle. Differences in hormonal regulation of the endometrium may result in different gene expression patterns. In normal endometrium and eutopic endometrium from women with endometriosis, as seen from our immunohistochemistry results, expression of TNS1 during the proliferative and secretory phases was unchanged. Further investigation of endometriotic tissues confirmed that TNS1 mRNA and protein levels did not vary significantly between the proliferative and secretory phases. Thus, TNS1 expression in the tissues and serum of women with endometriosis appears to be stable across the menstrual cycle, indicating that TNS1 expression is unaffected by endogenous cyclic hormone changes but is influenced by exogenous hormonal changes, such as GnRHa treatment.

Our current investigation showed that duration of GnRHa treatment in women with endometriosis did not affect expression of TNS1 with regard to either mRNA or protein levels. Management of endometriosis with GnRHa treatment is based on its ability to induce amenorrhea and anovulation. Initially, during the first 3-4 weeks, the therapeutic response is characterized by an initial increase in gonadotropins and estradiol (referred to as a flare-up), followed by sustained hypoestrogenemia, amenorrhea and anovulation in the subsequent months.[27] Many studies have shown the effectiveness with which GnRHa induces regression of endometriosis and its clinical symptoms after 2-6 months of treatment.[27] Continuous exposure to GnRHa results in desensitization or downregulation of GnRH receptors, resulting in reduction of circulating serum gonadotropin levels and inhibition of ovarian hormone production. However, longer duration of GnRHa treatment is often associated with hypoestrogenic symptoms, including vasomotor instability, vaginal dryness and significant bone loss, which preclude its long-term use.[28]

The current study suggests that TNS1 has some functions of medical interest that may make it a potential target for therapeutic intervention in endometriosis and a biomarker for the effectiveness of GnRHa treatment of this disorder. Our results require further confirmation from a larger cohort of patients and extension to include groups of control women without endometriosis and women with different stages of endometriosis to explain further the role of TNS1 in disorder progression across endometriosis subtypes. The regulatory mechanisms of TNS1 in the pathogenesis of endometriosis by GnRHa also require further elucidation. Utilizing in vivo animal models to confirm the role of TNS1 in disorder progression would also be valuable in future studies.

5 | CONCLUSION

This study demonstrated a significant decrease in TNS1 expression in endometrial tissues and serum of women with endometriosis following GnRHa treatment. TNS1 expression also was stable across the menstrual cycle and independent of GnRHa treatment duration.

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CONFLICT OF INTEREST

The authors have no conflicts of interest in connection with the work reported herein.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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