**CEOG** Clinical and Experimental Obstetrics & Gynecology

# Expression and significance of ERβ and TrkB in endometriosis

**X. Yu[1], H. Ren[2], T. Liu[3], M. Yong[4], H. Zhong[3]**

*[1] Department of Gynecology, Dalian Obstetrics and Gynecology Hospital Affiliated to Dalian Medical University, Dalian*
*[2] Department of General Surgery of Dalian Friendship Hospital Affiliated to Dalian Medical University, Dalian*
*[3] Department of Pathology of Dalian Friendship Hospital Affiliated to Dalian Medical University, Dalian*
*[4] Dalian Medical University Graduate School, Dalian (China)*

**Summary**

*Objectives:* To study the potential pathogenesis of endometriosis (EMs) in an area of estrogen receptors (ERs) and tyrosine kinase receptor type B (TrkB) expressions in tissues from patients with EMs. *Study Design:* The authors examined the expressions of ERα, ERβ, TrkB, brain-derived neurotrophic factor (BDNF), and SGPL1 in tissues with EMs, using real-time PCR, western blot, and immunohistochemistry. *Results:* ERα and SGPL1 were mainly expressed in eutopic endometrium than that in ectopic endometrium of patients with ovarian endometriosis ($p < 0.05$), while ERβ, BDNF, and TrkB were adverse, mainly detected in ectopic endometrium of the same patients with EMs ($p < 0.01$ and $p < 0.05$ ) by real-time PCR and western blot. ERβ, ERα, TrkB, and SGPL1 proteins were mainly expressed in eutopic endometrium of proliferative phase with EMs than that in eutopic endometrium of secretory phase ($p < 0.05$ ). TrkB, BDNF, and SGPL1 were not found in endometrium of proliferative or secretory phase in control group. *Conclusions:* ERβ expressed in cytoplasm may mediate pathogenesis of EMs.

*Key words:* Endometriosis (EMs); Estrogen receptor-α (ERα); Estrogen receptor-β (ERβ); Tyrosine kinase receptor B (TrkB); Brain-derived neurotrophic factor (BDNF); Immunohistochemistry (IHC).

## Introduction

Endometriosis (EMs) is an estrogen-dependent disease characterized by the growth of endometrial stromal cells and glands outside of the uterine cavity. Between 1% and 7% of women in the general population [1] and up to 30% of women undergoing laparoscopy for chronic pelvic pain are diagnosed with endometriosis [2]. Although the cause of endometriosis remains an enigma, retrograde menstruation of shed endometrial cells and tissue fragments is thought to be central to the development of this disease. However, regurgitation of menstrual effluent occurs to some degree in all women of which only a fraction develop endometriosis. Hence, factors other than access of endometrial contents to the pelvis via retrograde menstruation are thought to contribute to the pathogenesis of this disease. However, the critical event(s) or biochemical change(s) that ultimately lead to the establishment of endometriosis remain unknown.

Most recently reported that ovarian endometriosis and adenomyosis result from the physiological mechanism of 'tissue injury and repair' involving local estrogen production in an estrogen-sensitive environment normally controlled by the ovary [3].

It is well known that the development and progression of endometriosis depends on the presence of estrogen. The classical human estrogen receptor, ERα,was cloned in 1986, and a second estrogen receptor, ERβ, was cloned from human testis in 1996. Both ERα and ERβ act as transcription factors and are believed to play key roles in growth regulation of the endometrium and endometriosis. Previous reports have demonstrated markedly higher levels of ERβ and lower levels of ERα in endometriotic tissues and endometriotic stromal cells. Differences in the ERα/ERβ ratio between endometriotic and endometrial stromal cells could have important functional implications, since these ERs have different ligand binding characteristics [4].

Endometriosis also is undoubtedly multifactorial in origin involving features of immune modulation, adhesion [5. 6], angiogenesis [7, 8], invasion, proliferation, and decreased apoptosis [9, 10]. Several distinct lines of evidence suggest that endometrial cells destined to become endometriotic implants are biochemically and functionally distinct from eutopic endometrium of women without endometriosis. Tyrosine kinase receptor B (TrkB) gene expression, a neurotrophic factor receptor expressed in the brain and ovary, has recently been identified in deep infiltrating endometriosis by gene array [11]. TrkB is thought to be important in resistance to anchorage independent apoptosis (ANOIKIS) and thus could be important in the pathogenesis of endometriosis. ANOIKIS (detachment-induced apoptosis) has recently been proposed in cancer biology. Tumor cells sensitive to ANOIKIS undergo apoptosis on entering blood or lymphatic vessels after disseminating away from a primary tumor. ANOIKIS resistant tumor cells do not undergo apoptosis in such an environment and thus

Revised manuscript accepted for publication August 5, 2014

show more aggressive tumorigenic and metastatic phenotype. Neurotrophins and their tyrosine kinase receptors, tropomyosin related kinase (Trk) regulate the proliferation, differentiation, and death of neuronal cells, and have been implicated in the pathogenesis and prognosis of neuroblastomas. The expression of brain derived neurotrophic factor (BDNF) and its tyrosine kinase receptor, TrkB has been correlated with clinical outcome and chemotherapy resistance in neuroblastoma. Aside from neuroblastoma, the BDNF/TrkB pathway has been shown to have an important role in a number of human malignancies such as ovarian cancer [12], Wilms' tumor, prostate cancer, lung cancer, pancreatic carcinoma, and hepatocellular carcinoma.

In the culture of male rat-hypothalamic neurons, E2 induced an increase in the levels of TrkB. Additional experiments showed that when the E2-dependent increase of TrkB was prevented by using an antisense against mRNA for TrkB, the axogenic effect of E2 was suppressed, indicating a convergence of the signaling pathways for E2 and neurotrophins [13, 14]. Also interesting result has been reported that both mRNA and protein levels of TrkB were increased dose dependently in response to FSH treatment, implicating that FSH is one of the putative upstream mediators for TrkB expression in ovarian cancer cells [15].

Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid metabolite involved in cancer development through stimulation of cell survival, proliferation, migration, and angiogenesis. Irreversible degradation of S1P is catalyzed by S1P lyase (SPL). The human SGPL1 gene that encodes SPL maps to a region often mutated in cancers [16].

For the first time, in the present study, eutopic endometrium and ectopic endometrium of patients with endometriosis were analyzed for ERs, ANOIKIS suppressor TrkB, and SGPL1 expression at mRNA and protein levels. To further probe potential mechanism for potential pathogenesis of endometriosis, revealing correlation expression of ERβ with TrkB in EM with ovarian endometriosis or adenomyosis.

## Materials and Methods

*Patients*

Patients undergoing laparoscopy for endometriosis with ovarian endometriosis or adenomyosis were recruited for this study beginning in May 2012 in the Dalian Obstetrics and Gynecology Hospital. All tissue samples were obtained with full and informed patient consent. All patients did not receive hormonal treatments, such as GnRH agonist or sex steroids, and did not use intrauterine contraception for ≥ six months before surgery. Eighteen cases of recruited patients had regular menstrual cycles (between 26 and 32 days), had their menstrual history confirmed, and had serum 17β E2 and P levels measured just before surgery. The endometrial dating criteria as described by Noyes *et al.* [5] in 1950 and the menstrual history were used to assess the menstrual cycle phase. Endometrial tissue biopsies were performed just before operation. Finally, a total of 18 patients (nine patients during the proliferative phase, nine patients during the secretory phase) were selected for the present study. All patients had complaints of pain or infertility before surgery. Samples of ovarian endometriosis and adenomyosis tissues (three patients had ovarian endometriosis and adenomyosis at the same time), and the matched endometrium were divided into two portions. One part was fixed in 10% formalin, embedded in paraffin and stained with hematoxylin-eosin for pathological diagnosis. The other portion was frozen in liquid nitrogen immediately after removal and stored until use. Twelve patients with ectopic pregnancy or hysteromyoma who had tubal resection or hysteromyoma excision or uterectomy was the control group. The study protocol followed the ethical guidelines of Dalian Friendship Hospital. Informed consent was obtained from all subjects.

*RNA isolation and real-time PCR*

Total RNA was prepared by using TRIzol. Primers for cDNA amplification were as follows: ERα (F- TCTGCCAAGGAGACTCGCTA, R-CTTTTCGTATCCCACCTTTCAT 241bp), ERβ (F-GATGAGGGGAAATGCGTAGAAG, R-GGCAATCACCCAAACCAAAG 234bp), TrkB (F- TTACCCGAAACAAACTGACGA, R- GTCTGGACTGGATTTAGCCTCTT, 146bp), BDNF (F- TGGAGGCTATGTGGAGTTGG, R- GGGCATAAGTCGGCTTGAGT 257bp), SGPL1 (F- TCGGTGAGAACGGCTATGT, R- CGGGCGTGTAGTAATGTGAT 247bp), and GAPDH (F-GGGA AACTGTGGCGTGAT, R- GAGTGGGTGTCGCTGTTGA 299bp). Real-time PCR was used using SYBR Premix Ex Taq. All reactions were carried out according to the manufacturer's protocols. The primer sequences were as above. The annealing temperature for these primer sets was 60°C. The specificity of each PCR reaction was confirmed by melting curve analysis. The level of target gene expression in each sample was normalized to the respective GAPDH expression level [17].

*Immunohistochemistry*

Tissue samples were fixed, cut, mounted, deparaffinized and rehydrated. For endogenous peroxidase quenching, the slides were incubated in 0.3% hydrogen peroxide ($H_2O_2$) for ten minutes. After blocked with goat serum, the sections were incubated with the primary antibody solution at 4°C overnight (1:100 dilution for ERα, 1:70 dilution for TrkB, 1:100 dilution for BDNF, and 1:200 dilution for SGPL1, and 1:200 dilution for ERβ). The slides were rinsed twice with PBS and incubated with peroxidase-conjugated secondary antibodies for one hour at room temperature. After rinsing, color was developed by incubating slides with three, 3-diaminobenzidine for five minutes followed by counterstaining with hematoxylin for ten seconds. The slides were dehydrated with successive washes of $dH_2O$, 95% and 100% ethanol, and xylene before mounting with cover slips. The breast cancer and ovarian cancer specimens were used as positive control, while the primary antibodies was replaced with PBS as negative control. Immunohistochemical staining results were evaluated with blind method. Nuclear immunoreactivity with ERα, nuclear, and cytoplasmic immunoreactivity with ERβ, cytomembrane, and cytoplasmic immunoreactivity with TrkB, and cytoplasmic immunoreactivity with BDNF and SGPL1 were considered as positive.

*Western blot analysis*

After rinsed twice with ice cold PBS, the cells were scraped and lysed in ice-cold HNTG buffer [50 mmol/L HEPES (pH 7.5), 150 mmol/L NaCl, 10% glycerol, 1% Triton X-100, 1.5 mmol/L $MgCl_2$, one mmol/L EDTA, ten mmol/L sodium PPI, 100 μmol/L sodium orthovanadate, 100 mmol/L NaF, ten μg/mL aprotinin, ten μg/ml leupeptin, and one mmol/L phenylmethylsulfonyl fluoride] on ice for 30 minutes. Total proteins were measured Bio-Rad protein assay reagent according to the manufacturer's protocol. Twenty μg protein was separated on 12% of SDS-PAGE gels and transferred to nitrocellulose membrane. After blocked with 10%

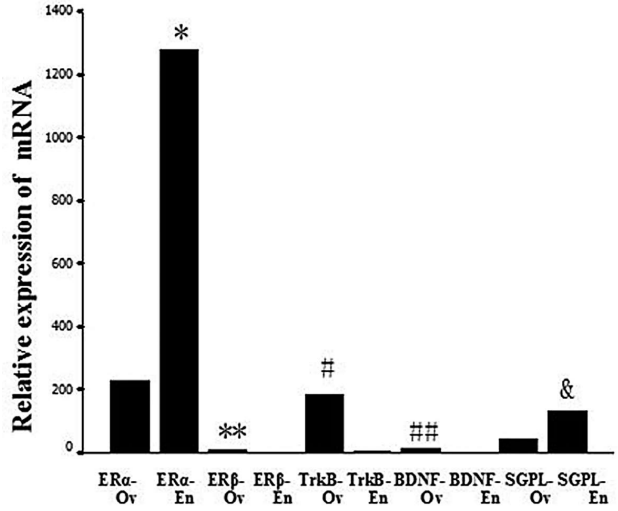


Figure 1. — Expression levels of ERα, ERβ, TrkB, BDNF, and SGPL1 mRNA in ectopic and eutopic endometrium of patients with ovarian endometriosis were determined by real-time PCR. GAPDH was used as an internal control. Data are expressed as the mean; bars, ±SD. *$p < 0.05$ ERα mRNA of ectopic endometrium versus eutopic endometrium; **$p < 0.01$ ERβ mRNA of ectopic endometrium versus eutopic endometrium; #$p < 0.05$ TrkB mRNA of ectopic endometrium versus eutopic endometrium; ##$p < 0.01$ BDNF mRNA of ectopic endometrium versus eutopic endometrium; &$p < 0.05$ SGPL1 mRNA of ectopic endometrium versus eutopic endometrium. (Ov represents ectopic endometrium; En represents eutopic endometrium).

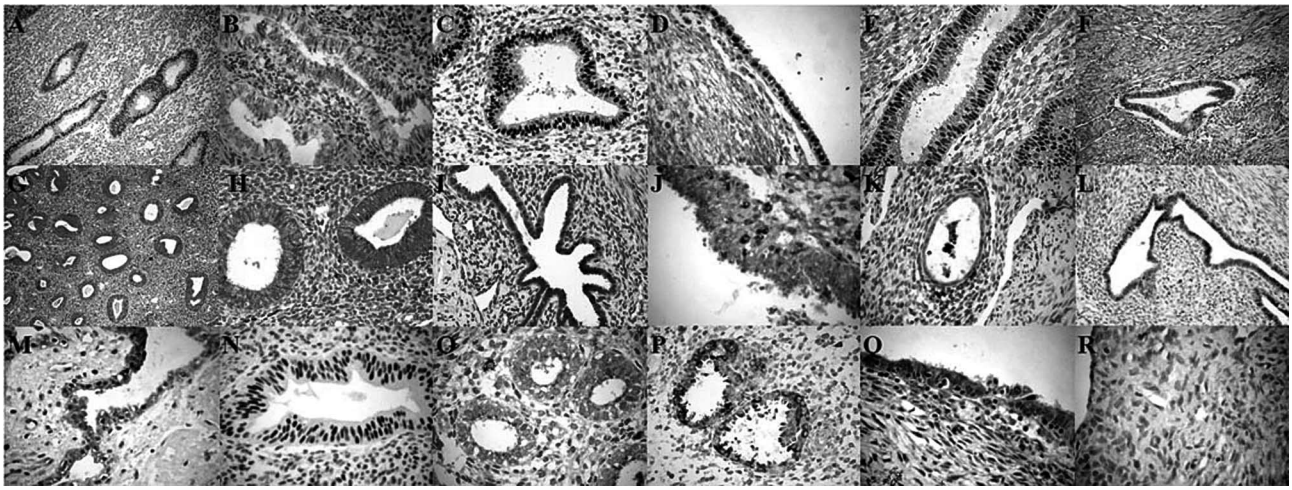


Figure 2. — Expressions of ERα and ERβ proteins of patients with ovarian endometriosis or adenomyosis were determined by IHC. (A) Nuclear staining of ERα protein was detected in eutopic endometrium of proliferative phase. (B) Nuclear staining of ERα protein was detected in eutopic endometrium of proliferative phase, the higher power. (C) ERα protein was detected in eutopic endometrium of secretory phase. (D) Nuclear staining of ERα protein was detected in ectopic endometrium of ovarian endometriosis. (E) Nuclear staining of ERα protein was detected in ectopic endometrium of adenomyosis. (F) Nuclear staining of ERα protein was detected in ectopic endometrium of adenomyosis, at lower power. (G) Cytoplasmic staining of ERβ protein was detected in eutopic endometrium of proliferative phase. (H) Cytoplasmic staining of ERβ protein was detected in eutopic endometrium of proliferative phase, at higher power. (I) ERβ protein was detected in eutopic endometrium of secretory phase. (J) Cytoplasmic staining of ERβ protein was detected in ectopic endometrium of ovarian endometriosis. (K) Cytoplasmic staining of ERβ protein was detected in ectopic endometrium of adenomyosis. (L) Cytoplasmic staining of ERβ protein was detected in ectopic endometrium of adenomyosis, at lower power. (M, N) Nuclear staining of ERα protein was detected in endometrium of proliferative and secretory phase. (O, P) Cytoplasmic staining of ERβ proteinm was detected in endometrium of of proliferative and secretory phase. (Q) Nuclear staining of ERα protein was detected in ovarian cortex. (R) Cytoplasmic staining of ERβ protein was detected in ovarian cortex (Maximal magnification ×400).

bovine serum albumin in 1×Tris-buffered saline, the membrane was incubated with various primary antibody against ERα, TrkB, BDNF, SGPL1, ERβ, and GAPDH at 4°C overnight. The membrane was washed with PBS three times, and then incubated with peroxidase-linked secondary antibody (1:10000) for one hour at room temperature. The signals were developed with the ECL kit, scanned, and analyzed with TotalLab software. The relative expression of target proteins was presented as the ratio to β-actin or GAPDH.

*Statistics*

Results are presented as mean ± SD. Data were analyzed with paired Student's *t* test for comparison between groups. *A* $p < 0.05$ was considered statistically significant.

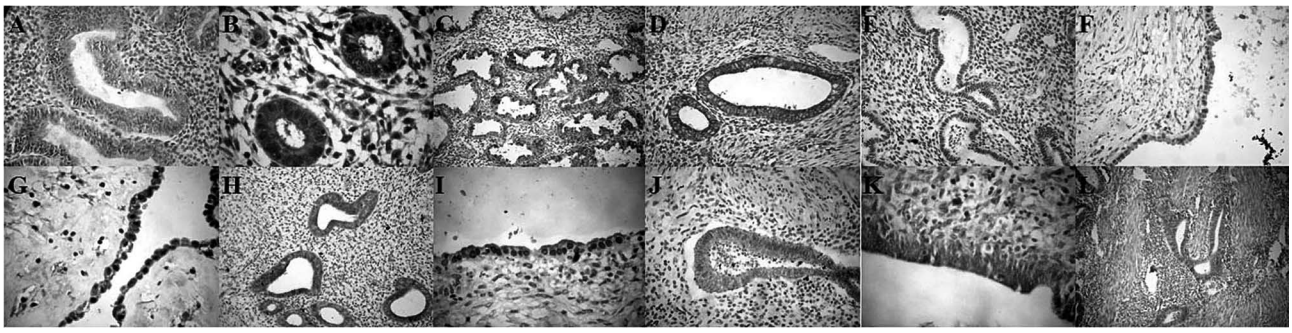


Figure 3. — Expressions of TrkB, BDNF, and SGPL1 proteins of patients with ovarian endometriosis or adenomyosis were determined by IHC. (A, B) Cytoplasmic staining of TrkB protein was detected in eutopic endometrium of proliferative phase. (C) Cytoplasmic staining of TrkB protein was detected in eutopic endometrium of proliferative phase, at lower power. (D) TrkB protein was detected in eutopic endometrium of secretory phase. (E) TrkB protein was not detected in eutopic endometrium of secretory phase. (F, G) Cytoplasmic staining of TrkB protein was detected in ectopic endometrium of ovarian endometriosis. (H) Cytoplasmic staining of TrkB protein was detected in functionalis glands of adenomyosis. (I) Cytoplasmic staining of BDNF protein was detected in ectopic endometrium of ovarian endometriosis. (J) Cytoplasmic staining of BDNF protein was detected in functionalis glands of adenomyosis. (K) Cytoplasmic staining of SGPL1 protein was detected in ectopic endometrium of ovarian endometriosis. (L) Cytoplasmic staining of SGPL1 protein was detected in functionalis glands of adenomyosis (maximal magnification ×400).

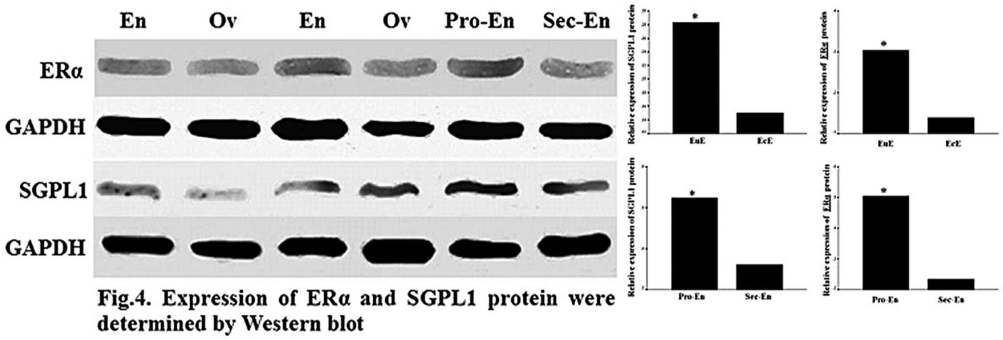


Figure 4. — Expressions of ERα and SGPL1 proteins in ectopic and eutopic endometrium of patients with ovarian endometriosis were determined by western blot. Western blot analysis shows the bands demonstrating ERα protein (66 kDa) and SGPL1 protein (63 kDa) in ectopic endometrium, eutopic endometrium, and proliferative or secretory phase of eutopic endometrium of patients with ovarian endometriosis differently $*p < 0.05$. (Ov and ECE represent ectopic endometrium; En and EuE represent eutopic endometrium. Pro-En represent eutopic endometrium of proliferative phase, Sec-En represent eutopic endometrium of secretory phase).

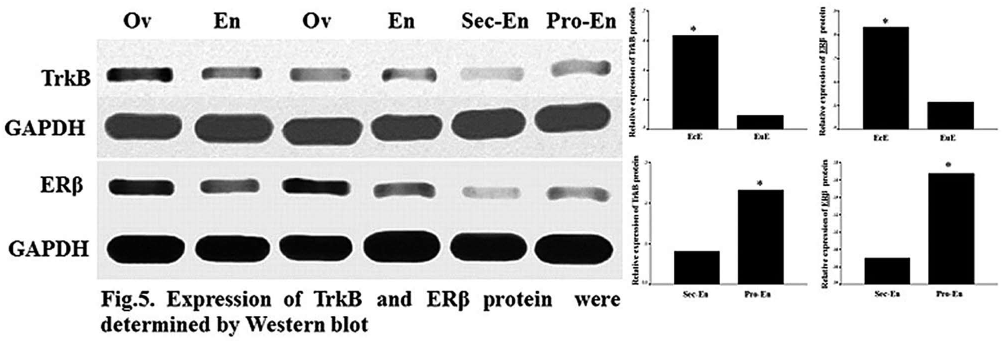


Figure 5. — Expressions of ERβ and TrkB proteins in ectopic and eutopic endometrium of patients with ovarian endometriosis were determined by western blot. Western blot analysis shows the bands demonstrating TrkB protein (145kDa) and ERβ protein (59 kDa) in ectopic endometrium, eutopic endometrium, and proliferative or secretory phase of eutopic endometrium of patients with ovarian endometriosis differently $*p < 0.05$. (Ov and ECE represent ectopic endometrium; En and EuE represent eutopic endometrium. Pro-En represent eutopic endometrium of proliferative phase, Sec-En represent eutopic endometrium of secretory phase).

## Results

*Expression of ERα, ERβ, TrkB, BDNF, and SGPL1 in eutopic and ectopic endometrium of patients with ovarian endometriosis*

The authors observed ERα and SGPL1 mRNAs were mainly expressed in eutopic endometrium than that in ectopic endometrium of the same patient with ovarian endometriosis ($p < 0.05$). ERβ, BDNF, and TrkB mRNAs were notably detected in ectopic endometrium than that in corresponding eutopic endometrium ($p < 0.01$ and $p < 0.05$ ) by real-time PCR. The authors also found high ratios of ERβ mRNA and ERα mRNA levels in endometriotic tissues of ovarian endometriosis (Figure 1). At protein level, certificated that ERα and SGPL1 proteins were dominantly expressed in eutopic endometrium, while ERβ and TrkB proteins were detected mainly in ectopic endometrium by western blot ($p < 0.05$ ) (Figure 4). Importantly ERβ, ERα, TrkB, and SGPL1 proteins were mainly expressed in eutopic endometrium of proliferative phase with ovarian endometriosis than that of secretory phase by western blot ($p < 0.05$ ) (Figures 4 and 5). TrkB, BDNF, and SGPL1 were not found in proliferative or secretory phase endometrium of control group (data not shown). ERα and ERβ were expressed in proliferative or secretory phase endometrium of control group normally.

*Expression of ERα, ERβ, TrkB, BDNF and SGPL1 proteins in eutopic and ectopic endometrium of patients with EMs by IHC*

IHC staining showed that nuclear staining of ERα and cytoplasmic staining of ERβ in eutopic and ectopic endometrium, while TrkB, BDNF, and SGPL1 proteins were detected cytoplasmic staining in eutopic and ectopic endometrium of patients with EMs. Especially the authors certified that ERβ, ERα, TrkB and SGPL1 proteins were mainly expressed in eutopic endometrium of proliferative phase with ovarian endometriosis or adenomyosis by IHC (Figures 2 and 3). In addition, the present study demonstrated positive staining of ERα, ERβ, TrkB, BDNF, and SGPL1 proteins in functionalis glands of adenomyosis.

## Discussion

Endometriosis is a benign lesion with malignant ability, and the incidence reaches up to 80% in infertile women. EMs may be associated with localized high concentration of estrogen and abnormal enhancement of ANOIKIS suppression and migration, but the mechanism is not yet elucidated. Although the exact mechanism for the development of endometriosis remains unclear, there is a large body of research data and circumstantial evidence that suggests a crucial role of estrogen in the establishment and maintenance of this disease.

Despite its sensitivity to estrogen, endometriosis appears to contain a unique but severely altered complement of steroid hormone receptors compared with that of its normal tissue counterpart, eutopic endometrium. Moreover, a number of investigators have reported markedly elevated levels of estrogen receptor ERβ and lower levels of ERα in human endometriotic tissues when compared with eutopic endometrial tissues [19]. The present results also revealed that higher ratios of ERβ and ERα mRNA levels in ectopic endometrium than that in eutopic endometrium from ovarian endometriosis patients by real time PCR.

Unlike ERα, ERβ plays a minor role in mediating estrogen action in the uterus, on the hypothalamus/pituitary, the skeleton, and other classic estrogen target tissues. However, a clear role for ERβ has been established in the ovary, cardiovascular system, and brain as well as in several animal models of inflammation including endometriosis [20]. The high ratios of ERβ and ERα levels in endometriotic stromal cells in turn lead to increased ERβ binding to the PR promoter and mediates the downregulation of expression of PR [21].

In light of endometriosis's malignant behaviors, the authors detected expression of SGPL1 and TrkB which may relate to oncogenesis of human cancer in endometriosis tissues.

By affecting S1P metabolism and the expression of Bcl-2 members, the loss of SPL enhances cell resistance to anticancer regimens and results in an increased ability of cells to acquire a transformed phenotype and become malignant [22]. For the first time the authors found that SGPL1 was expressed in endometrium of patients with EMs, as ERα, the higher level was in eutopic endometrium than that in ectopic endometrium of ovarian endometriosis. There is fewer research regarding expression of TrkB in EMs reported. Expression of TrkB was found upregulated of TrkB in epithelial cells from deep infiltrating endometriosis (DIE), which might be involved in molecular mechanisms of perineural and intraneural invasion [11]. A study detected TrkB mRNA in epithelial cells from ovarian endometriosis patients [23]. The expression levels of TrkB in epithelial cells from DIE were significantly decreased in patients with preoperative GnRH agonist or progestin. However, the functional roles of TrkB in DIE remain to be clarified [24]. In this study, for the first time the authors found that expression of TrkB mRNA and protein and its ligand BDNF was detected in ectopic and eutopic endometrium tissues from ovarian endometriosis patients, and the higher levels were revealed in ectopic endometrium than that in eutopic endometrium notably by real time PCR and western blot.

To certificate the results above, the authors examined expression of ERα, ERβ, TrkB, BDNF, and SGPL1 proteins in eutopic and ectopic endometrium from patients with ovarian endometriosis or adenomyosis by IHC. IHC staining showed that nuclear staining of ERα and cytoplasmic staining of ERβ in epithelial cells, and cytoplasmic staining of TrkB, BDNF, and SGPL1 protein in epithelial cells of eutopic and ectopic endometrium with ovarian en-

dometriosis or adenomyosis. Whether there are differences in the expressions of some proteins through the menstrual cycle in uteri with and without EMs or adenomyosis is a interesting topic. For the first time the authors revealed that ERβ, ERα, TrkB, and SGPL1 proteins were mainly expressed in eutopic endometrium of proliferative phase other than secretory phase with ovarian endometriosis or adenomyosis, and TrkB, BDNF, and SGPL1 were not found in proliferative or secretory phase endometrium of control group (data not shown). This result confirmed that distinct property in eutopic endometrium of women with EMs, and the molecular changes in eutopic endometrium of proliferative phase would more crucial for endometrial cells destined to become endometriotic implants of women with endometriosis.

The present study also demonstrated that nuclear or cytoplasmic staining of ERα, ERβ, TrkB, BDNF, and SGPL1 proteins were detected in functionalis glands of adenomyosis [25]. It is reported that the ERα expression in the adenomyotic endometrium was different from that of the normal endometrium and the foci in the mid secretory phase of the cycle, but expression of ERα in the inner and outer myometrium was not statistically significantly different. The ERβ expression was statistically significantly elevated in the adenomyotic functionalis gland during the proliferative phase and throughout the myometrium across the entire menstrual cycle.

In humans, however, the roles of ERα and ERβ on proliferation and apoptosis in endometrium and endometriosis are not well known; ERα regulation of cell cycle in eutopic endometrium is probable. High levels of ERβ suppress ERα expression and response to estradiol in endometrial and endometriotic stromal cells via binding to classic and nonclassic DNA motifs in alternatively used ERα promoters. ERβ also regulates cell cycle progression and might contribute to proliferation of endometriotic stromal cells [18]. The present results showed the similar expression pattern of ERβ and ANOIKIS suppressor TrkB in EMs. Unlike ERα, ERβ showed nuclear ataining by IHC. The significance of this cytoplasmic staining is not yet clear, however, the earlier findings on ERα may provide an analogical interpretation for localization of ERβ in cytoplasm or/and vesicle: a subpopulation of ERα was localized in caveolae [26]; antiapoptotic signal was mediated by ERα when ERα was localized on the membrane, while this signal was lost when ERα was in the nucleus [27]. Recently, this signaling above mediated by membrane associated ERα and ERβ has been termed nongenomic signaling and more recently nonnuclear signaling [28, 29]. ERα and ERβ are found both in association with the plasma membrane (not transmembrane), in the cytoplasm and in the nucleus. Estrogen bound membrane associated ERα and ERβ can each activate a signaling cascade that includes PI3K and Akt, as well as ERK 1/2, JNK and p38. This signaling cascade protects the cell from injury, except for JNK, which increases apoptosis. Interestingly in this study the author used breast cancer and ovarian cancer specimens as positive control for ERα and ERβ protein detection (IHC), ERα was not observed in these specimens; ERβ was demonstrated with nuclear staining in ovarian carcinoma, and cytoplasmic and membranous staining in breast carcinoma simultaneously.

Full-length TrkB is a 145kDa transmembrane protein, preferentially activated by brain-derived neurotrophic factor (BDNF). Following ligand binding, TrkB forms homodimers resulting in auto-phosphorylation on tyrosine residues, which is required for its catalytic and signaling activities. TrkB also could be auto-phosphorylated independent of BDNF stimulation in ovarian cancer cells, with the PI3K/AKT activation mediated by TrkB overexpression, the ovarian cancer cells showed high ability of chemotherapy-resistance and metastasis [12].

Although few studies assessed the relationship between E2/ERs and TrkB/BDNF expression in cancers or EMs, but a clue was revealed by Cheung *et al.* [30]: FSH is one of the putative upstream mediators for TrkB expression in ovarian cancer cells. If it could be presumed that the nonnuclear signaling of E2/ERβ activation in EMs may upregulate TrkB expression, with the PI3K/AKT activation mediated by TrkB overexpression, the endometriotic cells showed high ability of proliferation, ANOIKIS suppression, and invasion. Moreover, the potential mechanism of FSH/E2 inducing TrkB expression and activation mediated by the nonnuclear signaling of E2/ERs needs to be further investigated.

## Acknowledgements

This work was supported by the Key Project of the Dalian Science and Technology Bureau, Grants 2009E12SF144.

## References

[1] Barbieri R.L.: "Etiology and epidemiology of endometriosis". *Am. J. Obstet. Gynecol.*, 1990, *162*, 565.
[2] Howard F.M.: "The role of laparoscopy in chronic pelvic pain: promise and pitfalls". *Obstet. Gynecol. Surv.*, 1993, *48*, 357.
[3] Leyendecker G., Wildt L., Mall G.: "The pathophysiology of endometriosis and adenomyosis: tissue injury and repair". *Arch. Gynecol. Obstet.*, 2009, *280*, 529.
[4] Xue Q., Lin Z., Cheng Y.H., Huang C.C., Marsh E., Yin P., *et al.*: "Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis". *Biol. Reprod.*, 2007, *77*, 681.
[5] Nisolle M., Casanas-Roux F., Donnez J.: "Early-stage endometriosis: adhesion and growth of human menstrual endometrium in nude mice". *Fertil. Steril.*, 2000, *74*, 306.
[6] Beliard A., Noel A., Goffin F., Frankenne F., Foidart J.M.: "Role of endocrine status and cell type in adhesion of human endometrial cells to the peritoneum in nude mice". *Fertil. Steril.*, 2002, *78*, 973.
[7] Akoum A., Metz C.N., Al-Akoum M., Kats R.: "Macrophage migration inhibitory factor expression in the intrauterine endometrium of women with endometriosis varies with disease stage, infertility status, and pelvic pain". *Fertil. Steril.*, 2006, *85*, 1379.
[8] Kharfi A., Boucher A., Akoum A.: "Abnormal interleukin-1 receptor type II gene expression in the endometrium of women with endometriosis". *Biol. Reprod.*, 2002, *66*, 401.

[9] Béliard A., Noël A., Foidart J.M.: "Reduction of apoptosis and proliferation in endometriosis". *Fertil. Steril.*, 2004, *82*, 80.
[10] Braun D.P., Ding J., Shen J., Rana N., Fernandez B.B., Dmowski W.P.: "Relationship between apoptosis and the number of macrophages in eutopic endometrium from women with and without endometriosis". *Fertil. Steril.*, 2002, *78*, 830.
[11] Matsuzaki S., Canis M., Vaurs-Barrière C., Pouly J.L., Boespflug-Tanguy O., Penault-Llorca F., *et al.*: "DNA microarray analysis of gene expression profiles in deep endometriosis using laser capture microdissection". *Mol. Hum. Reprod.*, 2004, *10*, 719.
[12] Yu X., Liu L., Cai B., He Y., Wan X.: "Suppression of anoikis by the neurotrophic receptor TrkB in human ovarian cancer". *Cancer Sci.*, 2008, *99*, 543.
[13] Spencer-Segal J.L., Tsuda M.C., Mattei L., Waters E.M., Romeo R.D., Milner T.A., *et al.*: "Estradiol acts via estrogen receptors alpha and beta on pathways important for synaptic plasticity in the mouse hippocampal formation". *Neuroscience*, 2012, *202*, 131.
[14] Aguirre C.C., Baudry M.: "Progesterone reverses 17beta-estradiol-mediated neuroprotection and BDNF induction in cultured hippocampal slices". *Eur. J. Neurosci.*, 2009, *29*, 447.
[15] Cheung A.N., Siu M.K., Au C.W., Chan H.Y., Wong E.: "Follicle stimulating hormone can act on receptors of other growth hormone". *Carcinogenesis*, 2007, *28*, 2060.
[16] Colié S., Van Veldhoven P.P., Kedjouar B., Bedia C., Albinet V., Sorli S.C., *et al.*: "Disruption of sphingosine 1-phosphate lyase confers resistance to chemotherapy and promotes oncogenesis through Bcl-2/Bcl-xL upregulation". *Upregulation Cancer Res.*, 2009, *69*, 9346.
[17] Li X., Cao X., Li X., Zhang W., Feng Y.: "Expression level of insulin-like growth factor binding protein 5 mRNA is a prognostic factor for breast cancer". *Cancer Sci.*, 2007, *98*, 1592.
[18] Elkas J., Armstrong A., Pohl J., Cuttitta F., Martínez A., Gray K.: "Modulation of endometrial steroid receptors and growth regulatory genes by tamoxifen". *Obstet. Gynecol.*, 2000, *95*, 697.
[19] Trukhacheva E., Lin Z., Reierstad S., Cheng Y.H., Milad M., Bulun S.E.: "Estrogen receptor (ER) beta regulates ERalpha expression in stromal cells derived from ovarian endometriosis". *J. Clin. Endocrinol. Metab.*, 2009, *94*, 615.
[20] Xiu-li W., Wen-jun C., Hui-hua D., Su-ping H., Shi-long F.: "ERB-041, a selective ERβ agonist, inhibits iNOS production in LPS-activated peritoneal macrophages of endometriosis via suppression of NF-γB activation". *Mol. Immunol.*, 2009, *46*, 2413.
[21] Karita M., Yamashita Y., Hayashi A., Yoshida Y., Hayashi M., Yamamoto H., *et al.*: "Does advanced-stage endometriosis affect the gene expression of estrogen and progesterone receptors in granulosa cells?" *Fertil. Steril.*, 2011, *95*, 889.
[22] Colié S., Van Veldhoven P.P., Kedjouar B., Bedia C., Albinet V., Sorli S.C., *et al.*: "Disruption of sphingosine 1-phosphate lyase confers resistance to chemotherapy and promotes oncogenesis through Bcl-2/Bcl-xL upregulation". Cancer Res., 2009, *69*, 9346.
[23] Matsuzaki S., Canis M., Pouly J.L., Botchorishvili R., Déchelotte P.J., Mage G.: "Differential expression of genes in eutopic and ectopic endometrium from patients with ovarian endometriosis". *Fertil. Steril.*, 2006, *86*, 548.
[24] Matsuzaki S., Canis M., Pouly J.L., Botchorishvili R., Déchelotte P.J., Mage G.: "Both GnRH agonist and continuous oral progestin treatments reduce the expression of the tyrosine kinase receptor B and mu-opioid receptor in deep infiltrating endometriosis". *Hum. Reprod.*, 2007, *22*, 124.
[25] Mehasseb M.K., Panchal R., Taylor A.H., Brown L., Bell S.C., Habiba M.: "Estrogen and progesterone receptor isoform distribution through the menstrual cycle in uteri with and without adenomyosis". *Fertil. Steril.*, 2011, *95*, 2228.
[26] Matsuyama S., Ohkura Y., Eguchi H., Kobayashi Y., Akagi K., Uchida K., *et al.*: "Estrogen receptor beta is expressed in human stomach adenocarcinoma". *J. Cancer Res. Clin. Oncol.*, 2002, *128*, 319.
[27] Chambliss K.L., Yuhanna I.S., Mineo C., Liu P., German Z., Sherman T.S., *et al.*: "Estrogen receptor alpha and endothelial nitric oxide synthase are organized into a functional signaling module in caveolae". *Circ. Res.*, 2000, *87*, E44.
[28] Kousteni S., Bellido T., Plotkin L.I., O'Brien C.A., Bodenner D.L., Han L., *et al.*: "Sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptionalactivity". *Cell*, 2001, *104*, 719.
[29] Cabilla J.P., Nudler S.I., Ronchetti S.A., Quinteros F.A., Lasaga M., Duvilanski B.H.: "Nitricoxide-sensitive guanylyl cyclase is differentially regulated by nuclear and non-nuclear estrogen pathways in anterior pituitary gland". *PLoS One*, 2011, *6*, e29402. doi: 10.1371/journal.pone.0029402. Epub 2011 Dec 28..
[30] Cheung A.N., Siu M.K., Au C.W., Chan H.Y., Wong E.: "Follicle stimulating hormone can act on receptors of other growth hormone". *Carcinogenesis*, 2007, *28*, 2060.

Address reprint requests to:
X. YU, M.D.
Department of Gynecology, Dalian Obstetrics and Gynecology Hospital
Affiliated to Dalian Medical University
No. 33 Dunhuang Road
Dalian, 116033 (China)
e-mail: yuxiaohui369@163.com