Elevated immunoreactivity to tissue factor and its association with dysmenorrhea severity and the amount of menses in adenomyosis

Xishi Liu†, Jichan Nie†, and Sun-Wei Guo*
Shanghai OB/GYN Hospital, Fudan University, Shanghai 200011, China
*Correspondence address. Shanghai Obstetrics and Gynecology Hospital, Fudan University Shanghai College of Medicine, 419 Fangxie Road, Shanghai 200011, China. Tel: +86-21-5388-2377; Fax: +86-21-X; E-mail: hoxa10@gmail.com
Submitted on July 26, 2010; resubmitted on October 7, 2010; accepted on October 12, 2010

BACKGROUND: Heavy menstrual bleeding and dysmenorrhea are two top complaints from women with symptomatic adenomyosis, yet their etiology is poorly understood. Tissue factor (TF) has been shown to be upregulated in endometriosis and at the endometrial bleeding sites of women with long-term progesterin only contraception. We sought to investigate the expression and localization of TF in eutopic and ectopic endometrium of women with adenomyosis and in endometrium of women without adenomyosis. We also sought to determine the relationship, if any, between TF immunoreactivity and the amount of menses, uterus size and severity of dysmenorrhea.

METHODS: We retrieved tissue samples of eutopic and ectopic endometrium from 50 women with adenomyosis and of endometrium from 18 women without adenomyosis. The tissue sections were subjected to immunostaining and microscopic evaluation to assess the presence and localization of TF in both proliferative and secretory phases in both eutopic and ectopic endometrium and normal endometrium. Information on the amount of menses, severity of dysmenorrhea and other information were collected.

RESULTS: We found that TF immunoreactivity was significantly increased in both eutopic and ectopic endometrium as compared with normal endometrium. In addition, we found that the elevated TF immunoreactivity is associated with heavy menses and increased severity of dysmenorrhea.

CONCLUSIONS: These results suggest that TF is involved in adenomyosis-associated heavy menstrual bleeding and dysmenorrhea and thus may be a potential therapeutic target in treating symptomatic adenomyosis and perhaps also chronic pelvic pain in women with adenomyosis.

Key words: adenomyosis / heavy menstrual bleeding / immunohistochemistry / tissue factor / severity of dysmenorrhea

Adenomyosis is a common gynecologic disorder with a poorly understood pathogenesis (Bergeron et al., 2006). Besides a soft and diffusely enlarged uterus, its symptoms also include dysmenorrhea, heavy menstrual bleeding and subfertility (Farquhar and Brosens, 2006). Treatment of adenomyosis has been a challenge, with hysterectomy being the treatment of choice (Bergeron et al., 2006). Although the disease is hormone-sensitive (Kitawaki, 2006), progestogenic agents are not very effective, and gonadotropin-releasing hormone (GnRH) agonists induce suppression of adenomyosis yet their use is restricted by short duration (Bergeron et al., 2006). In addition, the symptoms often recur after discontinuation of GnRH agonists therapy (Grow and Filer, 1991).

Tissue factor (TF) is a cell membrane-bound glycoprotein and a member of the cytokine receptor superfamily (Versteeg et al., 2001). TF is constitutively expressed by a diverse array of extravascular cells, especially in many important organs and tissues such as heart, brain, testis, placenta and kidney, but not in liver and skeletal muscle. Following vascular injury, TF binds to FVII/VIIa and the TF:FVIIa complex activates prothrombin to thrombin, triggering the clotting cascade. Besides its role in coagulation, TF also functions in many biological processes such as hemostasis, thrombosis, inflammation, angiogenesis and tumor growth (Versteeg et al., 2008). In normal endometrium, TF has been shown to be expressed mainly in...
stromal cells of the secretory phase and TF transcription has been shown to be regulated by progesterone, SP1 and SP3 (Krikun et al., 1998; Krikun et al., 2000). Animal studies also show that TF is required for uterine hemostasis and gestation (Erlich et al., 1999).

Krikun and his associates have shown that TF expression is elevated in eutopic and ectopic endometrium in women with endometriosis (Krikun et al., 2008). They also show that protease-activated receptor 2 (PAR2), a putative TF receptor thought to regulate intracellular signaling, is also upregulated in the eutopic endometrium (Krikun et al., 2008). This seems to echo the report that PAR2 activation stimulates proliferation and interleukin (IL)-6 and IL-8 secretion in endometriotic stromal cells (Hirota et al., 2005), and is consistent with the fact that TF:FVlla complex activates PAR2 (Rao and Pendurthi, 2005). These data, taken together, suggest that TF is involved in the pathogenesis of endometriosis, possibly in angiogenic and inflammatory signaling (Krikun et al., 2008). The elevated TF expression also has been reported by Lockwood and his associates) to be involved in abnormal uterine bleeding in women with long-term use of progesterin only contraception (LTPOC) (Runic et al., 2000), possibly due to the angiogenic role of TF (Lockwood et al., 2009).

Adenomyosis and endometriosis share remarkable similarities in definition, their estrogen-dependence, symptomatology, treatment modality and many documented molecular aberrations (Ota et al., 1998; Maia et al., 2005). In addition, the two often occur concurrently (Kunz et al., 2005, 2007), even though which proceeds is unclear. These similarities strongly suggest that certain common culprits may be involved in the pathogenesis of both conditions. Indeed, it has been proposed recently that both endometriosis and adenomyosis may result from tissue injury and repair (Leyendecker et al., 2009).

We hypothesized that, as in endometriosis, TF expression is also elevated in adenomyosis, and may be responsible for heavy menstrual bleeding and dysmenorrhea in adenomyosis. Hence, in this study we sought to investigate the expression and localization of TF in eutopic and ectopic endometrium of women with adenomyosis and in endometrium of women without adenomyosis. We also sought to determine the relationship, if any, among the amount of menses, uterus size and severity of dysmenorrhea and TF immunoreactivity. Finally, we sought to evaluate the correlation, if any, between TF immunoreactivity and other proteins reported previously to be aberrantly expressed in adenomyosis.

**Materials and Methods**

**Patients**

The patients recruited to this study were reported previously (Nie et al., 2009, 2010a,b). Briefly, 50 women with histologically confirmed adenomyosis (excluding endometriosis) seen at Shanghai OB/GYN Hospital, Fudan University Shanghai Medical College, from 2004 to 2005, were recruited for this study. All of them had diffuse adenomyosis, the most common subtype seen in China. Their diagnoses were made by transvaginal ultrasound before surgery and histologically confirmed post-operatively, based on the presence of endometrial glands and stroma at least one lower-power field of view (about 10 x 10, or about 2–3 mm) away from the endometrial-myometrial junction (Rosai, 1989).

All patients’ ectopic, along with their homologous eutopic, endometrial tissue samples, were collected after hysterectomy and fixed in 10% buffered formalin and routinely processed for paraffin embedding. For controls, after informed consent, we collected endometrial tissue samples through curettage from 18 women with surgically diagnosed benign ovarian cysts, but none had endometriosis, adenomyosis or myoma. The selection of the controls was based on menstrual phase and age as well as disease status.

All women in both study and control groups were premenopausal and had regular menses (range of the menstrual cycle lengths: 21–35 days), with no history of hormone therapy or intratine device use for ≥6 months prior to the surgery or tissue collection. The menstrual phase in which the patient was at the time of surgery was determined based on the day elapsed since the last period. All endometrial samples were grouped either in proliferative (Days 1–14 of the cycle) or secretory (Days 15–28 of the cycle) phase. In both the study and control groups, exactly half of women were in the proliferative phase while the other half were in the secretory phase. Depending on whether they changed their sanitary pads <3, between 3 and 6 or >6 times a day, respectively, as we reported previously (Nie et al., 2009, 2010a,b), their amount of menses during menstruation was grouped into three classes: light, moderate and heavy. The severity of dysmenorrhea was classified as mild, moderate and severe, as reported previously (Nie et al., 2009, 2010a,b) and roughly equivalent to the verbal descriptor scale.

For each patient with adenomyosis, information was collected, through reading medical charts and interviewing patients, on age at surgery, uterus size (calculated as πD1D2D3/6, where D1 = the distance from fundus to the internal os of the cervix, D2 = transverse diameter at the level of the cornua and D3 = anteroposterior diameter at the level of cornua), complaint of dysmenorrhea, severity of dysmenorrhea (none, mild, moderate or severe), duration of dysmenorrhea, amount of menses (light, moderate or heavy) and gravidity. This study was approved by the institutional ethics review board of Shanghai OB/GYN Hospital.

**Tissue samples, antibodies and immunohistochemistry**

 Archived, formalin-fixed, paraffin-embedded tissue blocks were retrieved from the Department of Pathology, Shanghai OB/GYN Hospital. Serial 4-μm sections were obtained from each block, with the first resultant slide being stained for H&E to confirm pathologic diagnosis, and the subsequent slides stained for TF. Routine deparaffinization and rehydration procedures were performed.

The mouse monoclonal antibody against TF (ab17375, Abcam, Cambridge, UK) diluted to 1:200 was used as the primary antibody. For antigen retrieval, the slides were heated at 98°C in an EDTA buffer (pH 9.0) for a total of 45 min and cooled naturally to the room temperature. Sections were then incubated with the primary antibody overnight at 4°C. After slides were rinsed, they were incubated with biotinylated secondary antibody, Supersensitive TM Universal (anti-mouse) detection reagent (horse-radish peroxidase) (G5500705, Shanghai Gene Tech Company, Shanghai), at room temperature for 30 min. The bound antibody complexes were stained for 3–5 min or until appropriate for microscopic examination with diaminobenzidine and then counterstained with hematoxylin (for 30 s) and mounted.

A negative control was also incorporated using preimmune immunoglobulin G (IgG) instead of the primary antibody. The scoring of the immunoreactivity was evaluated by digital image analysis using the Image Pro-Plus 6.0 (Media Cybernetics, Inc., Bethesda, MD, USA) as reported previously (Wang-Tilz et al., 2006) without prior knowledge of any of the clinico-pathological information. Briefly, images were obtained with the microscope (Olympus BX51, Olympus, Tokyo, Japan) fitted with a digital camera (Olympus DP70, Olympus, Tokyo, Japan). A series of 10 random images on several sections were taken for each immunostained parameter to obtain a mean value. Staining was defined via colour
intensity, and a colour mask was made. The mask was then applied equally to all images, and measurements were obtained. Immunohistochemical parameters assessed in the area detected included integrated optical density (IOD), total stained area (S) and (c) mean optical density (MOD), which is defined as MOD = IOD/S, equivalent to the intensity of stain in the positive cells. In the following, the immunoreactivity level or staining level thus referred to the MOD values.

Data analysis
For descriptive statistics, we used boxplot (Tukey, 1977) to graphically depict groups of immunoreactivity data, in which the bottom and top of the box represent the lower and upper quartiles, respectively, the band near the middle of the box represents the median, and the ends of the whiskers represent the smallest and the largest non-outlier observations. The dots outside the box, if any, are outliers. The comparison of distributions of continuous variables between or among two or more groups was made using the Wilcoxon test and Kruskal-Wallis test, respectively. Jonckheere-Terpstra trend test was used to test for trend of increasing TF immunoreactivity in women who complained of dysmenorrhea of various severity. Pearson’s or Spearman’s rank correlation coefficient was used when evaluating correlations between two variables when both variables are continuous or when at least one variable is ordinal. The relationship between various clinical and pathological parameters was compared with chi-square tests.

To evaluate which factors are associated with the severity of dysmenorrhea, we used the Cox regression model for the discrete or grouped survival time data (Kalbfleisch and Prentice, 1980). Here, we assumed that the severity of dysmenorrhea can progress through various stages, hence patients start with ‘none’ or ‘mild’ and can deteriorate to ‘moderate’ or even ‘severe’ and are unlikely to reverse this progression. With this model, we can treat the severity as if they were discrete ‘survival time’ without censoring (Liu and Guo, 2008). Note, however, that a longer ‘survival time’ actually means a more severe condition of dysmenorrhea, whereas the other 30 (60%) and 19 (38%) patients complained of moderate and heavy menses, respectively. No relationship was found between the severity of dysmenorrhea and the amount of menses (Spearman’s rank correlation r = 0.15, P = 0.31), between severity of dysmenorrhea and the uterus size (r = 0.19, P = 0.19), nor between uterus size and the amount of menses (r = 0.07, P = 0.64). The time elapsed from the first diagnosis of adenomyosis to surgery ranged from 0.25 to 144 months, with a median length of 36 months or 3 years.

TF immunohistochemistry in normal, eutopic and ectopic endometrium
Consistent with the previous report (Krikun et al., 2008), weak staining of TF was seen in mostly stromal cells but also glandular epithelial cells of the normal endometrium of secretory phase, while no staining of TF was seen in the proliferative endometrium in controls, and the difference was statistically significant (P = 0.034). In eutopic and ectopic endometrium, in contrast, intense TF staining was found mainly in membranes and cytoplasm of glandular epithelial cells, but could also be found in the nucleus in some cases (Fig.1). This is also consistent with the finding in endometriosis (Krikun et al., 2008). There was no difference in TF staining between the proliferative and secretory phases (both P-values > 0.09). Unlike endometriosis in which the highest TF expression was seen in ectopic endometrium (Krikun et al., 2008), no difference in TF staining level was found between eutopic and ectopic endometrium (P = 0.34, paired comparison; Fig. 2).

The immunoreactivity to TF in both eutopic and ectopic endometrium was significantly higher than that in the normal endometrium (P = 7.6 × 10^-5 and P = 0.003, respectively; Fig. 2). In fact, the TF staining in eutopic and ectopic endometrium was significantly correlated (r = 0.45, P = 0.001). Taken together, these data suggest that TF is overexpressed in both eutopic and ectopic endometrium in women with adenomyosis, similar to endometriosis as reported by Krikun et al. (2008).

Amount of menses and TF immunohistochemistry
Since TF is involved in coagulation and is shown to be involved in abnormal uterine bleeding resulting from LTPOC (Runic et al., 2000), we next examined the relationship, if any, between TF immunoreactivity and amount of menses. Since there was only one woman who reported light menses, we divided all cases into two groups: women who reported light and moderate menses, and those reported heavy menses. We found that TF staining in eutopic endometrium was significantly higher in women with heavy menses than those with light/moderate menses (P = 0.013; Fig. 3). TF staining in ectopic endometrium was also higher in the women with heavy menses, but the difference did not reach statistical significance (P = 0.26). This suggests that TF overexpression in eutopic endometrium is associated with, and possibly responsible for, heavy menstrual bleeding in women with adenomyosis.

Severity of dysmenorrhea and TF immunohistochemistry
Since dysmenorrhea is another major complaint in women with adenomyosis (Matalliotakis et al., 2003), we next attempted to examine as whether TF immunohistochemistry is associated with the severity of dysmenorrhea in women with adenomyosis. We found that TF
staining levels in both eutopic and ectopic endometrium were significantly correlated with the severity of dysmenorrhea (Spearman’s \( r = 0.51, P = 0.001 \) and \( r = 0.31, P = 0.029 \), respectively). The Jonckheere-Terpstra test revealed that TF immunoreactivity in eutopic and ectopic endometrium was significantly associated with the severity of dysmenorrhea (\( P = 0.0003 \) and 0.043, respectively; Fig. 4) with increased staining in the more severe cases.

A multivariate analysis using the Cox regression model for discrete survival data, involving TF staining in eutopic and ectopic endometrium along with patients’ demographic variables such as age, menstrual phase in which the tissue sample was harvested, number of live births, number of pregnancies, size of the uterus and the amount of menses, indicated that TF staining (\( P = 0.004 \)) in eutopic endometrium is the only factor that is associated with the severity of

**Figure 1** Immunohistochemical staining of TF. (A) Staining in a normal endometrial tissue, using preimmune IgG, instead of the primary, antibody, that serves as a negative control. (B) Immunohistochemical staining in a normal endometrial tissue. (C) and (D) Staining in epithelial cells of eutopic and ectopic endometrium from a woman with adenomyosis. All magnifications were ×200. Scale bars represent 100 μm.
dysmenorrhea, while other factors had no effect. The proportional odds model yielded an identical result.

**Uterus size and TF immunohistochemistry**

As enlarged uterus is one of three major presentations of adenomyosis after abnormal uterine bleeding and dysmenorrhea (Matalliotakis et al., 2003), we also examined the relationship between uterus size and TF immunohistochemistry. However, no correlation was found (all P-values > 0.05).

**TF immunoreactivity and expression of other previously reported proteins**

We also examined, in the same type of tissues, the relationship of immunoreactivity to TF with other proteins that we have reported previously, since these correlation coefficients may reveal a possible inherent relationship between the two proteins that is otherwise obscure. We found that in both eutopic and ectopic endometrium, TF expression correlated positively with the microvascular density (MVD) (r = 0.46 and 0.43, P = 0.0008 and 0.0018, respectively, in eutopic and ectopic endometrium), suggesting that TF may indeed be involved with angiogenesis in adenomyosis. This seems to be further supported by the correlation of TF expression with that of both SLIT and ROBO1 (r = 0.50 and r = 0.39, P = 0.0002 and 0.005, respectively, for SLIT and ROBO1 in ectopic endometrium), which are found to be elevated in adenomyosis and are likely to be involved in angiogenesis (Nie et al., 2010a, b). In addition, TF expression correlated with that of NF-kB p52 subunit in eutopic endometrium and p65 subunit in ectopic endometrium (r = 0.38 and r = 0.31, P = 0.004 and 0.023, respectively).
Discussion

Among women with symptomatic adenomyosis, excessively heavy menstrual bleeding and dysmenorrhea are the top two complaints (Peric and Fraser, 2006). Yet the molecular mechanisms as to how adenomyosis causes the two conditions are largely unclear. There are reports that heavy bleeding and dysmenorrhea are both positively associated with the depth of penetration of individual adenomyotic glands into the myometrium and with the density, on histological inspection, of deep endometrial glands with the myometrium (Bird et al., 1972; Nishida, 1991; McCausland and McCausland, 1998; Levger et al., 2000; Cirpan et al., 2008), but other studies report that adenomyosis-related symptoms are variable, non-specific and related to other associated pathological conditions (Nikkanen and Punnonen, 1980; Kilkku et al., 1984). What determines the frequency and severity of dysmenorrhea in adenomyosis remains a conundrum (Peric and Fraser, 2006). Few biomarkers for heavy bleeding or dysmenorrhea or its severity in adenomyosis have been identified. Without these biomarkers, targeted treatment remains a challenge, as is the case now.

We have previously reported that nuclear p65 immunoreactivity is positively associated with heavier menses, and decreased progesterone receptor-B and increased nuclear p65 immunoreactivity in ectopic endometrium are significantly associated with the severity of dysmenorrhea in women with adenomyosis (Nie et al., 2009). We have also reported that immunoreactivity to oxytocin receptor (OTR) and transient receptor potential vanilloid type 1 (TRPV1) were both positively correlated with the severity of dysmenorrhea, and found to be significant predictors for dysmenorrhea severity (Nie et al., 2010a,b). These putative biomarkers for heavy bleeding and/or dysmenorrhea in adenomyosis offer the first, yet important, glimpse into the possible molecular mechanisms underlying adenomyosis-related heavy bleeding and dysmenorrhea. The identification of more biomarkers for adenomyosis-associated heavy bleeding and/or dysmenorrhea should help identify therapeutic targets and more effective, targeted treatment.

TF is known to play an important role in hemostasis and, in gynecology, TF is involved in the pathogenesis of endometriosis, possibly in angiogenic and inflammatory signaling (Krikun et al., 2008). Elevated TF expression has also been reported to be involved in abnormal uterine bleeding in women with LTPOC (Runic et al., 2000). While TF is known to be involved in coagulation, it is interesting, and somewhat surprising, to see that TF levels in endometrial bleeding sites are actually higher than that in the non-bleeding sites (Runic et al., 2000). The elevated TF expression is found to be accompanied by abnormally enlarged and distended blood vessels in the bleeding sites, suggesting an angiogenic role of TF (Krikun et al., 2009).

Our finding of increased endometrial TF expression in women with heavy menses as compared with those with light or moderate menses strongly suggest that, similar to abnormal uterine bleeding in the LTPOC case, TF may also be involved in heavy menstrual bleeding in women with adenomyosis. This postulation actually is further supported by the positive correlation of TF expression level with the MVD in both eutopic and ectopic endometrium, and with SLIT and ROBO1 expression in ectopic endometrium, since the SLIT and ROBO1 system has been reported to be involved in tumour angiogenesis (Wang et al., 2003; Wang et al., 2008) and apparently in angiogenesis in endometriosis (Shen et al., 2009). Incidentally, a positive correlation between TF expression and MVD has been reported in non-small-cell lung cancer (Koomagi and Volm, 1998) and prostate cancer (Abdulkadir et al., 2000). That said, we note that LTPOC-related abnormal uterine bleeding and adenomyosis-related heavy menstrual bleeding are pathologically different processes, with the former occurring from capillaries in progestin-modified endometrium at all stages of the cycle while the latter occurs mainly from the spiral arteries manifesting as a pathology of normal menstruation (Fraser and Hickey, 2000; Critchley et al., 2006). Future studies examining the TF expression in endometrial bleeding and non-bleeding sites in women with adenomyosis should provide more opportunity for unravelling the role of TF in adenomyosis-associated heavy bleeding.

While the association of TF overexpression and heavy menses may be expected, the correlation between TF expression level and the severity of dysmenorrhea may not be. It is possible, however, that increased uterine dysesthesia/contactility in adenomyosis (Kissler et al., 2006; Kunz et al., 2007), possibly due to elevated OTR expression as reported previously (Nie et al., 2010a,b), and its resulting mechanical stress induce TF expression (Houston et al., 1999), just as mechanical stretch resulting from contractility can stimulate IL-8 production in endometrial stromal cells (Harada et al., 2005). Incidentally, IL-8 also is overexpressed in LTPOC (Lockwood et al., 2009), and is likely to be expressed also in adenomyosis. Conversely, thrombin, which is downstream to TF in physiological conditions, can increase uterine contractility (O’Sullivan et al., 2004; Fitzgibbon et al., 2009). Thus, a feed-forward loop could be established, with elevated TF expression and increased contractility. The increased uterine contractility may be easily perceived as pain or cramps due to innervations (Zhang et al., 2009a,b) and/or elevated TRPV1 signaling (Nie et al., 2010a,b), as women with symptomatic adenomyosis are likely to experience central sensitization as in endometriosis (He et al., submitted for publication).

Our results provide evidence that, as in endometriosis (Krikun et al., 2008), TF expression is elevated in eutopic and ectopic endometrium. This adds another piece of evidence that endometriosis and adenomyosis share some important culprits (Nie et al., 2009) and gives credence to the proposal that endometriosis and adenomyosis may have similar pathogenesis (Leyendecker et al., 2009).

The identification of TF as a possible biomarker for adenomyosis-related heavy menstrual bleeding as well as dysmenorrhea suggests that TF may be a promising target for intervention. In fact, the finding that TF is involved in endometriosis led to the successful use of a novel chimeric immunoconjugate molecule (icon) to target endothelial TF in ectopic implants, resulting in devascularization and atrophy and the inhibition of ectopic implants in a nude mouse model of endometriosis (Krikun et al., 2010). Other drug candidates are also available. For example, andrographolide, an active ingredient extracted from an Chinese herb, has been shown to be a potent inhibitor of NF-κB p50 subunit (Wang et al., 2007), and also inhibits TF expression (Wang et al., 2007). Our own preliminary data suggest that andrographolides seem to have therapeutic value in treating adenomyosis, possibly by inhibiting p50 as well as TF expression (Liu et al., unpublished data).

Due to the cross-sectional nature of our study, we cannot say that TF is a specific enough target to be useful for the purpose of treating adenomyosis. What we have done is akin to the round-up of all suspects involved in a crime. Whether a particular suspect is a mastermind behind the crime, an executor, an aid or an abettor or merely
an unwilling follower coerced to participate in the crime will await further investigation. Yet given that the suspect has a history (TF is known to be involved in endometriosis and in angiogenesis in cancer), it is likely that TF is one of the major culprits in the pathogenesis of adenomyosis. The success in inhibiting ectopic implants in a nude mouse model of endometriosis by targeting endothelial TF in ectopic implants (Krikun et al., 2010) appears to support this view. Further research is warranted to see whether TF is truly a specific enough target for the treatment of adenomyosis.

For practical reasons, we could not harvest the endometrial tissue samples from basalis region of the endometrium in the controls. This, unfortunately, precludes the comparison in TF staining between the functionalis and the basalis. To be consistent, we only evaluated the TF expression in the functionalis in adenomyotic tissues. It is thus unclear as to whether the lack of a cyclical change in the TF expression in the adenomyotic tissues is due to the fact that basalis was not assessed.

The identification of TF involvement in adenomyosis may help to piece together some seemingly fragmented information on various signaling pathways involved in the pathophysiology of adenomyosis and/or endometriosis, as well as point out future research directions. A prominent event in TF signaling is activation of ERK/p38/JNK MAP kinases (Poulsen et al., 1998; Camer et al., 1999), which are known to be activated in endometriosis (Yoshino et al., 2004; Yoshino et al., 2006; Grund et al., 2008; Taniguchi et al., 2009; Zhang et al., 2010). It has been shown that, FVIIa, the natural TF ligand, induces the activation of the Src family members c-Src, Lyn and Yes, and subsequently PI3K, followed by stimulation of Akt/PKB as well as the small GTPases, Rac and Cdc42 (Versteeg et al., 2000). Rac then mediates p38 MAPK activation and cytoskeletal reorganization, whereas FVIIa-induced ERK stimulation requires PI3K enzymatic activity (Versteeg et al., 2000). Lyn expression has been reported to be dramatically in a rat model of endometriosis (Konno et al., 2007). Not surprisingly, PI3K and Akt/PKB are reported to be activated in endometriosis (Cinar et al., 2009; Laudanski et al., 2009). Yet ERK and Akt/PKB pathways are known to inhibit apoptosis (Bergmann et al., 1998; Dinnmeler and Zehner, 2000), and it is not surprising to note that endometriotic cells lose the ability to regulate cell-survival signaling (Zhang et al., 2009a,b) and are resistant to apoptosis (Johnson et al., 2005). Finally, as p21-activated kinase 1 (PAK1) is known to be a direct target of the small GTPases Rac and Cdc42 (Parrini et al., 2002), it is not surprising to see that PAK1 is overexpressed in both endometriosis (Kim et al., 2009) and adenomyosis (Kim et al., 2010). However, overexpression of Cdc42 has been reported in endometriosis, but not in adenomyosis (Goteri et al., 2006). Barring any error in the Cdc42 study (Goteri et al., 2006), it is possible that there may be some variations on the overall signaling theme in the context of adenomyosis. In addition, intracellular activators of PAK1 are not confined to small GTPases, and can include an array of molecules, such as PIX/COOL (Bagrodia et al., 1999). In principle, this discrepancy may be resolved by experiments examining the relationship among Rac1, Cdc42 and PAK1 simultaneously. It will also be of great interest in future studies to elucidate TF signaling and the roles, if any, of small GTPases (i.e. Ras GTPTase superfamily) in the pathophysiology of adenomyosis/endometriosis, especially in light of the reports of the involvement of Ras in the pathogenesis of endometriosis (Dinulescu et al., 2005; Matsuzaki et al., 2005; Tsuno et al., 2009).

In summary, this is, to our knowledge, the first study showing increased TF immunoreactivity in eutopic and ectopic endometrium in adenomyosis. In addition, we found that the elevated TF immunoreactivity is associated with heavy menses and increased severity of dysmenorrhea. These results suggest that TF is involved in adenomyosis-associated heavy menstrual bleeding and dysmenorrhea and thus may be a potential therapeutic target in treating symptomatic adenomyosis and perhaps chronic pelvic pain as well in women with adenomyosis.

Authors’ roles

J.C.N. carried out the entire experiment and participated in writing; X.S.L. participated in study design, patient recruitment and writing; S.W.G. conceived and designed the entire study, carried out data analysis and interpretation, and drafted the manuscript.

Acknowledgements

We thank two anonymous reviewers for their helpful comments on an earlier version of this manuscript.

Funding

This research was supported in part by grants 30872759 (S.W.G.) from the National Science Foundation of China, grants 074119517, 09PJ0105 and 10410700200 from the Shanghai Science and Technology Commission (S.W.G.), and grant (09-11) from the State Key Laboratory of Medical Neurobiology of Fudan University (S.W.G.).

References

Elevated tissue factor expression in adenomyosis


Rosai J. Female Reproductive System. Ackerman’s Surgical Pathology, St. Louis: Mosby, 1989.


