*Endocrine Journal* 2013, **60** (10), 1155-1164

**ORIGINAL**

# Possible involvement of nerve growth factor in dysmenorrhea and dyspareunia associated with endometriosis

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**Abstract.** Nerve growth factor (NGF) has been recently proposed as one of the key factors responsible not only for promotion of nerve fiber growth but also for the onset and maintenance of pain in a variety of diseases. The aim of this study was to investigate the role of NGF in the pelvic pain associated with endometriosis. Tissue and peritoneal fluid samples were collected from 95 women with laparoscopically and histopathologically confirmed endometriosis and 59 control women without endometriosis. Expression levels of NGF mRNA and protein were examined using real-time RT-PCR and immunohistochemistry, respectively. Concentration of NGF in the peritoneal fluid (PF-NGF) was measured using ELISA. The degree of dyspareunia and dysmenorrhea was evaluated using a verbal rating scale. Real-time RT-PCR analysis revealed that NGF mRNA was significantly more abundant in the ovarian endometriomas and peritoneal endometriosis than in the normal control endometrium. Immunohistochemical analyses demonstrated that NGF was prominently expressed and preferentially localized to the glands of the ovarian endometriomas and peritoneal endometriosis, whereas it was only weakly detectable in the normal endometrium. Although PF-NGF was undetectable in some normal subjects and endometriosis patients, elevated PF-NGF in the peritoneal fluid was more frequently observed in endometriosis patients with severe pain than in those with less severe pain. Our results suggest that NGF produced locally in the peritoneal cavity may be involved in the generation of endometriosis-associated pelvic pain.

*Key words*: Endometriosis, Nerve growth factor, Dysmenorrhea, Dyspareunia

**ENDOMETRIOSIS** is a common disorder of reproductive-age women. The prevalence of endometriosis is estimated to be 6-10% [1]. This benign disease mainly causes reduced fertility and several types of pain such as dysmenorrhea, deep dyspareunia, dyschezia, and chronic pelvic pain. Patient surveys of women in the United Kingdom and the United States have revealed that 15-20% and approximately 70% of patients present with infertility and pelvic pain including dysmenorrhea, respectively [2]. Acute and chronic pain compromises the quality of life in many patients with endometriosis and adenomyosis. Thus, pain relief is the primary treatment objective.

Molecular mechanism(s) underlying endometriosis-related pain have been extensively investigated [3-8]. Various humoral factors including prostaglandins and inflammatory cytokines locally produced in the lesions have been offered as causative factors for the pain. Nonsteroidal anti-inflammatory drugs (NSAIDs) are used as the first line therapy to treat chronic pelvic pain and dysmenorrhea through prostaglandin inhibition. NSAIDS are, however, occasionally ineffective in some endometriosis patients, particularly those with chronic pain. The mechanism(s) by which patients suffer from endometriosis pain, in particular, chronic pain remain elusive. Nerve growth factor (NGF) has been recently proposed as one of the key factors responsible not only for promotion of nerve fiber growth but also for the onset and persistence of chronic pain in a variety of tissues and diseases [9].

NGF is an essential factor for the development and maintenance of sensory and sympathetic neurons in the peripheral nervous system [10]. It also regulates the function of non-neuronal cells such as vas-

Submitted Jan. 17, 2013; Accepted Jun. 12, 2013 as EJ13-0027
Released online in J-STAGE as advance publication Jul. 24, 2013
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cular smooth muscle cells [11], immune cells [12, 13] and some tumor cells [14, 15]. In addition, NGF plays a crucial role in the generation of pain and hyperalgesia in a variety of acute and chronic pain states [9, 16, 17]. Indeed, treatment with a blocking antibody to NGF has been shown to effectively reduce pain and improve the quality of life in patients with tumor-induced bone cancer pain [18]. Recently, Anaf *et al*. have demonstrated that a prominence of NGF expression together with perineurial and endoneurial invasion are preferentially observed in deep adenomyotic nodules, the most symptomatic form of endometriosis [19]. Furthermore, Tokushige *et al*. have reported that peritoneal endometriotic lesions are intensely positive for NGF and possess significantly more nerve fibers than normal peritoneum [20].

In this study, we investigated whether there was an association between the generation or persistence of pain from endometriosis and NGF expression. We examined the expression and localization of NGF in the endometriotic lesions and measured the concentration of NGF in the peritoneal fluid (PF-NGF) of individual patients. Finally, we correlated the measured PF-NGF levels and the magnitude of pain in patients without and with endometriosis.

## Materials and Methods

### *Subjects and the collection of tissue and peritoneal fluid samples*

This study was approved by the Keio University Ethics Committee and all patients (range 32-45 years old) provided informed consent. Ovarian endometriomas ($n$=19) and peritoneal endometriotic lesions ($n$=8) at the uterine serosal surface, Douglas' pouch, bladder serosal surface, and uterosacral ligaments were obtained from consenting reproductive-aged patients who underwent laparoscopic surgery. Intrauterine endometrium samples ($n$=11) were obtained from women without endometriosis through hysterectomy for benign gynecological diseases such as leiomyomas. The collected tissues were divided into several aliquots and then fixed for pathological examinations, frozen in a Tissue Tek O.C.T. Compound (Sakura Finetechnical Co. Ltd., Tokyo, Japan) for immunohistochemistry, or snap-frozen for total RNA extraction. Peritoneal fluids were collected from 48 consenting, premenopausal patients without endometriosis (the menstrual phase; proliferative= 21, secretory= 12, and GnRH agonist treatment= 15) and 68 consenting premenopausal patients with endometriosis (proliferative= 37, secretory= 18, and GnRH agonist treatment= 13), at the time of laparoscopic surgery. Each subject was queried preoperatively regarding dyspareunia and dysmenorrhea and the response recorded using a verbal rating scale (VRS). The scales ranged from 0 to 3 where 0 indicated no pain, and 1, 2, and 3 were mild, moderate, and severe pain, respectively.

### *Semi-quantitative RT-PCR and real-time RT-PCR*

Total RNA was extracted from the tissue specimens using RNAiso reagent (TaKaRa Bio Inc., Shiga, Japan), and 5 μg of total RNA was then reverse-transcribed to first strand cDNA using Superscript III (Invitrogen Corp., Carlsbad, CA). An aliquot (1/100) of cDNA was then subjected to the PCR reaction with the specific primer pairs for *NGF*; 5'-ccaagggagcagctttctatcct-3' and 5'-agtgtcaagggaatgctgaagt-3', or for *36B4* as a housekeeping gene; 5'-atgcccagggaagacagggcgacc-3' and 5'-ttagtcgaagagaccgaatcccata-3', respectively. Reaction conditions were 35 cycles of denaturing at 94 ˚C for 20 sec, annealing at 55 ˚C for 30 sec and extending at 72 ˚C for 45 sec using Go Taq Master Mix (Promega Corp., Madison, WI). The PCR products were electrophoresed on a 2% agarose gel and subsequently visualized by ethidium bromide staining.

An aliquot (1/1000) of the cDNA was also subjected to real-time PCR using a LightCycler instrument (Roche Molecular Biochemicals, Mannheim, Germany) and SYBR Premix ExTaq (TaKaRa Bio Inc.). For quantifying NGF cDNA, the specific primer pairs 5'-ccaagggagcagctttctatcct-3' and 5'-agtgtcaagggaatgctgaagt-3' were used. The reaction conditions were 45 cycles of 10 sec denaturation at 95 ˚C, 10 sec annealing at 68 ˚C with a decrease in temperature of 0.5 ˚C every cycle for a secondary target annealing temperature of 58 ˚C, and 16 sec extension at 72 ˚C. Results were normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression, similarly quantified using primer pairs 5'-ggtgaaggtcggagtcaacgga-3' and 5'-gagggatctcgctcctggaaga-3' for 35 cycles of 5 sec denaturation at 95 ˚C, 5 sec annealing at 68 ˚C, and 7 sec extending at 72 ˚C. The PCR products were sequenced to confirm their identity. Data were analyzed using the 'Fit Point Method' in LightCycler software 3.3 (Roche Molecular Biochemicals). Relative quantification was made against serial dilution of GAPDH cDNA used as a housekeeping gene. The data presented are the mean

values from each sample group and the SEM.

***Immunohistochemistry***

Seven-micrometer thick sections were cut from the frozen tissue (intrauterine endometrium (n=3), ovarian endometriosis (n=4), endometriosis in the uterosacral ligament (n=1), and endometriosis on the peritoneal surface of uterus (n=2)), and mounted on glass slides. The sections were fixed in 4% paraformaldehyde, permeabilized by 2% Triton X-100, and endogenous peroxidase quenched with 0.3% $H_2O_2$. The sections were then incubated with TNBS (phosphate buffered saline containing 0.4% v/v of Triton X-100 and 1% v/v of normal horse serum) for 30 minutes at room temperature to block non-specific binding. After blocking, the sections were incubated with rabbit polyclonal NGF antibody (sc-548, Santa Cruz Biotechnology, Santa Cruz, CA), which was diluted in TNBS and used at 1:300 dilutions. After 1.5 h incubation at room temperature, the sections were stained using the Vectastain Peroxidase Rabbit IgG ABC Kit and DAB-Peroxidase Substrate Kit (Vector Laboratories Inc., Burlingame, CA) according to the manufacturer's recommended protocol. All slides were counterstained with hematoxylin, dehydrated, and mounted. For each immunostaining procedure, the primary antibody was replaced by non-immune rabbit serum as a negative control.

***Enzyme-linked Immunosorbent Assay (ELISA) for measurement of NGF concentrations in the peritoneal fluid***

To measure the concentrations of NGF in the peritoneal fluids of patients, we conducted assays using the DuoSet ELISA Development kit for human β-NGF (R&D systems, Minneapolis, MN) according to the manufacturer's recommended protocol. The working range was 20-2000 pg/mL. The collected peritoneal fluid samples were frozen and stored at –80˚C until the assays were carried out.

***Statistical analysis***

Statistical analysis of the mRNA expression levels was performed using a one-factor ANOVA and Tukey test. Differences in the distribution of endometriosis, VRS for dysmenorrhea, or VRS for dyspareunia were analyzed with respect to the levels of PF-NGF using the exact chi-square test. The differences were considered significant at $p<0.05$.

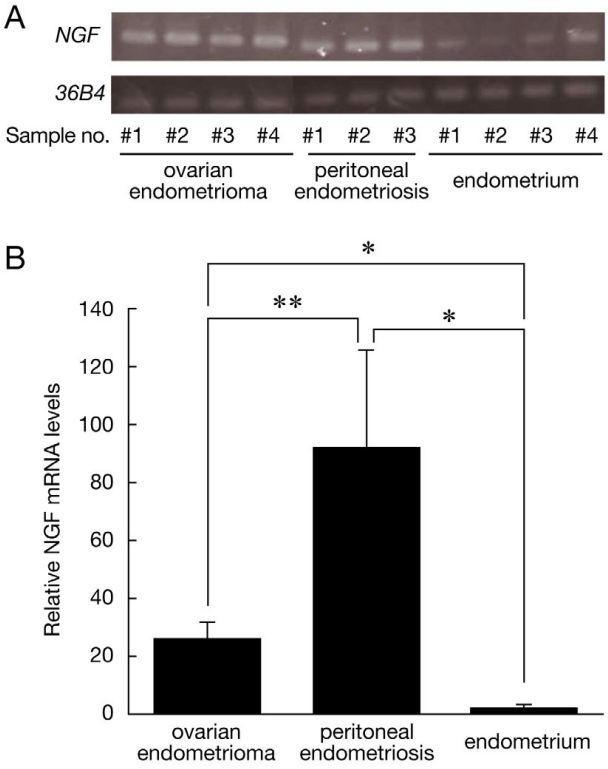


**Fig. 1** Up-regulation of NGF transcripts in endometriotic lesions (A) Representative images of semi-quantitative RT-PCR analysis of NGF and 36B4 mRNA derived from ovarian endometriomas (n=4), peritoneal endometriotic lesions (n=3), and eutopic endometrium (n=4). Three to four samples of total RNA extracted from each group of specimens were selected and subjected to semi-quantitative RT-PCR analysis for the specific amplification of NGF and ribosomal protein 36B4 as described in the *Materials and Methods*. The reaction mixtures were separated on a 2% agarose gel and visualized by ethidium bromide staining. #*n* indicates individual sample number. (B) Real-time RT-PCR analysis of NGF mRNA derived from ovarian endometrioma (n=19), peritoneal endometriotic lesions (n=8), and intrauterine endometrial tissues (n=11). These samples were first reverse-transcribed and subjected to real-time PCR experiments as described in the *Materials and Methods*. Each bar indicates the mean ± SEM of the relative NGF mRNA level. *, $p<0.05$; **, $p<0.01$.

## Results

We first determined with semi-quantitative RT-PCR whether NGF mRNA was up-regulated in the lesions of endometriosis. We found that the expression levels of NGF mRNA were higher in ovarian endometriomas and peritoneal endometriotic lesions than in intrauterine endometria without endometriosis (Fig. 1A). To obtain more quantitative data, we performed a real-

time RT-PCR analysis of NGF and GAPDH mRNA derived from 19 different ovarian endometriomas, eight peritoneal endometriotic lesions and 11 intrauterine endometrial samples without endometriosis. Relative NGF mRNA levels were determined by the ratio of NGF mRNA to GAPDH mRNA. As shown in Fig. 1B, relative NGF mRNA levels were significantly higher in ovarian endometriomas and peritoneal endometriotic lesions than in intrauterine endometrium ($p<0.05$). Furthermore, NGF transcripts were significantly more abundant in the peritoneal endometriotic lesions than in ovarian endometriomas ($p<0.01$). These results indicate that NGF mRNA is significantly up-regulated in endometriotic lesions.

To investigate the expression and localization of NGF protein, we next performed an immunohistochemical analysis of the intrauterine endometrium, ovarian endometriomas and peritoneal endometriosis with anti-NGF antibody. As shown in Fig. 2A, NGF protein was exclusively localized to the glandular epithelium of the intrauterine endometrium, but the staining intensity was quite low. In contrast, endometriotic lesions from the ovarian endometrioma showed strong NGF staining signals which were also localized to the glandular epithelium (Fig. 2B). Likewise, peritoneal endometriotic lesions collected from the uterosacral ligament and the uterine serosal surface exhibited similar immunostaining patterns (Figs. 2C and 2D, respectively). These results suggest that NGF protein is produced abundantly and specifically in endometriotic glandular cells.

We hypothesized that up-regulation of NGF mRNA and protein in endometriotic lesions was associated with the production and secretion of NGF from endometriotic lesions. To test this, we measured the concentration of NGF in the peritoneal fluids of patients with and without endometriosis. Out of 116 patients examined, peritoneal fluid NGF (PF-NGF) was undetectable in 48 patients. The other 68 patients with detectable PF-NGF had PF-NGF concentrations that ranged from 20 and 178 pg/mL. The lack of detectable NGF expression in some patients limited our ability to correlate NGF concentrations with corresponding VRS scores. Therefore, instead of using individual NGF levels, we set 28 pg/mL as the cut-off value for the NGF concentration. The value was determined based on the detection limit (20 pg/mL) of the ELISA used in this study, and on our preliminary test by using the several cut-off value (20 to 30 pg/mL) in the investigation of the relationship between the PF-NGF levels and VRS-based severity of dysmenorrhea and dyspareunia. We determined sensitivity value and specificity value for the relationship in each point, and then the Youden index (specificity+sensitivity-1, [21]) was calculated. At one of the cut-off values (28 pg/mL), the highest Youden index was obtained, and we therefore have determined 28 pg/mL as the cut-off value. The patients were then divided into the two groups with high ($28\leq$ pg/mL) and low (<28 pg/mL) PF-NGF. As shown in Fig. 3, the percentage of patients with high PF-NGF was higher in the group with endometriosis than in the group without endometriosis (30.9% *vs* 25.0%), but the difference was not significant.

NGF has been implicated in the generation and maintenance of pain in a variety of diseases [9, 16, 17]. Dysmenorrhea and dyspareunia are the two typical types of pain associated with endometriosis. To elucidate a possible involvement of NGF in endometriosis-associated pain, we investigated the relationship between the PF-NGF levels and VRS-based severity of dysmenorrhea and dyspareunia.

As shown in Fig. 4A, only 17.2% of patients with no pain (VRS=0) exhibited high PF-NGF levels, whereas 36-46% of patients with mild to severe pain had high levels. We therefore performed a subanalysis to statistically compare the proportion of patients with high PF-NGF in the group with no pain (VRS=0) with the group with mild to severe pain (VRS=1-3). The subanalysis revealed that there was a significant relationship between the frequency of elevated NGF levels and the severity of dysmenorrhea (Fig. 4B, $p<0.05$). Similarly, the proportion of patients with high PF-NGF was increased in patients with endometriosis in accordance with an increase in the VRS for dyspareunia (Fig. 5A). We performed a similar subanalysis and found that the proportion of high PF-NGF was significantly higher in patients with moderate or severe pain (VRS=2-3) than in those with no or mild pain (VRS=0-1) (Fig. 5B, $p<0.05$). Individual stages of endometriosis were not significantly associated with either the proportions of high PF-NGF patients or individual NGF concentrations (data not shown).

## Discussion

We have demonstrated in this study that NGF protein is preferentially localized to the glands in endometriotic lesions, which is consistent with previous reports [19, 20]. In rodent models of endometriosis, NGF expres-

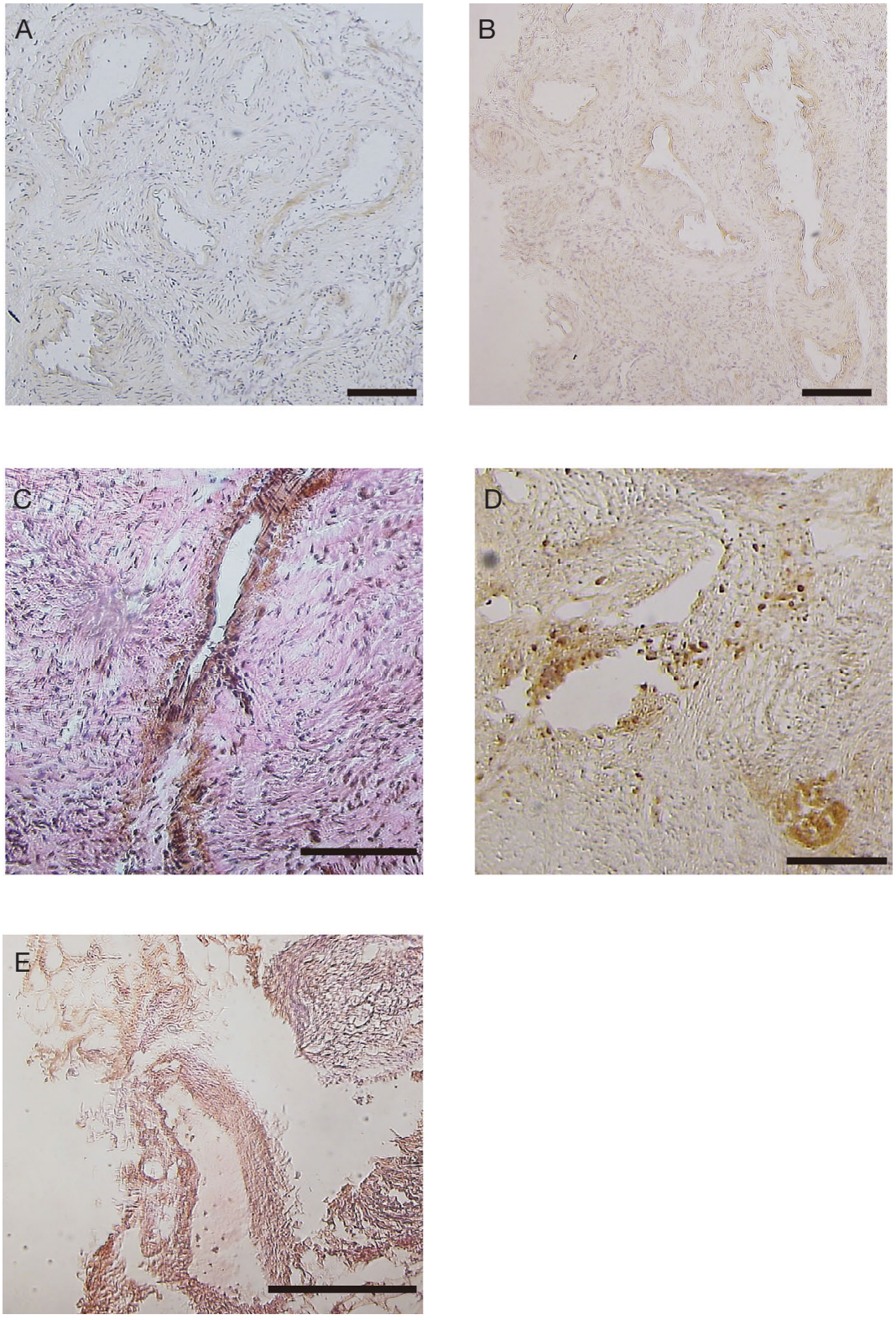


**Fig. 2** Up-regulation of the NGF protein and its preferential localization to the glandular epithelium in the endometriotic lesions Negative control staining image of the intrauterine endometrium is (A). Representative images of the immunohistochemical staining of intrauterine endometrium (B), ovarian endometriosis (C), endometriosis in the uterosacral ligament (D), endometriosis on the peritoneal surface of uterus (E). Scale bars, 100 μm.

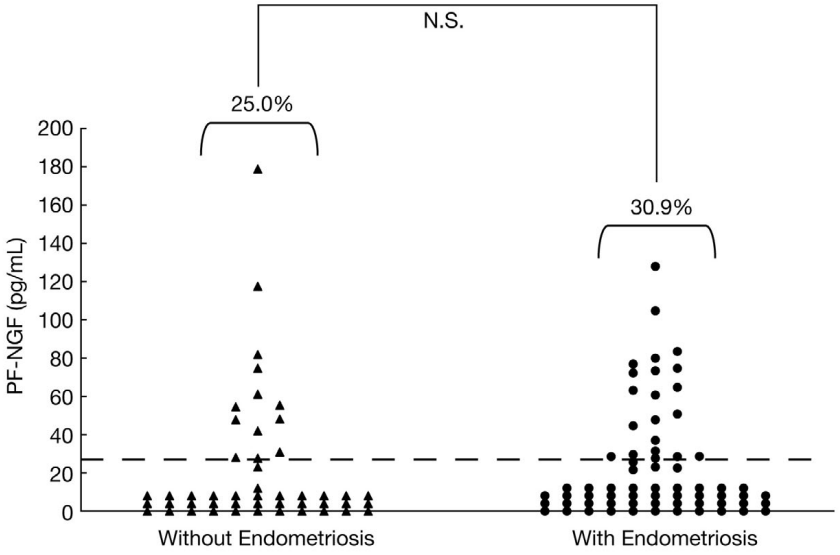


**Fig. 3** Relationship of the peritoneal fluid NGF (PF-NGF) level with the presence of endometriosis
The PF-NGF concentration of each patient without (closed triangle) or with (closed circle) endometriosis is dotted. The horizontal broken line indicates 28 pg/mL as the cut-off value. Twelve of 48 patients (25.0%) without endometriosis exhibited high level of PF-NGF (≥28 pg/mL), whereas 21 of 68 patients (30.9%) with endometriosis did. N.S., not significant.

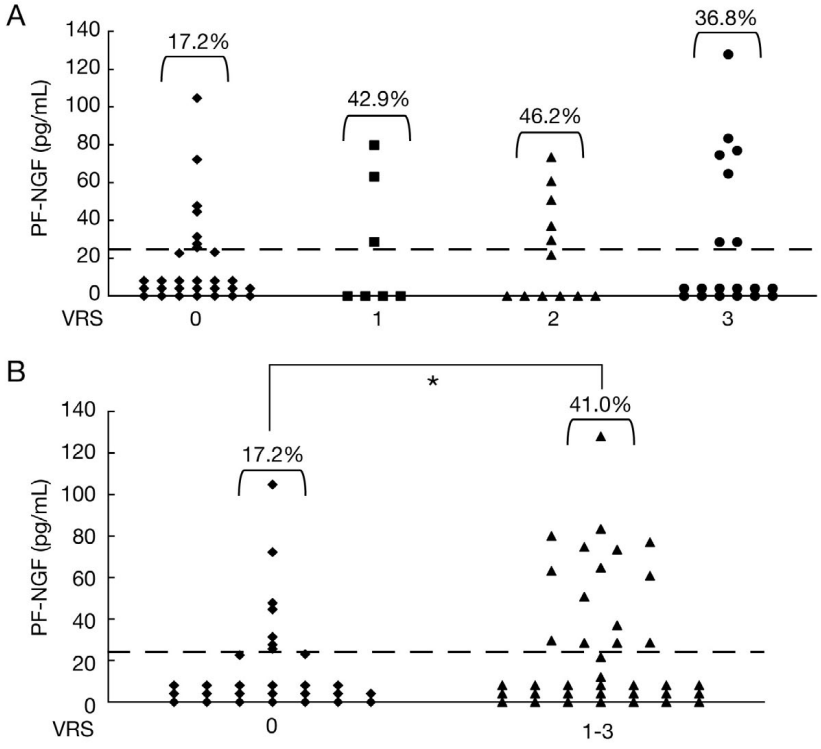


**Fig. 4** Relationship of the PF-NGF level and VRS for dysmenorrhea in patients with endometriosis
A, The PF-NGF concentration of each patient is dotted based on the VRS for dysmenorrhea. The horizontal broken line indicates 28 pg/mL as the cut-off value. Patients with high level of PF-NGF were found in 17.2 % (5/29) of patients with no pain (VRS=0, closed diamond), 42.9% (3/7) with mild pain (VRS=1, closed square), 46.2% (6/13) with moderate pain (VRS=2, closed triangle), and 36.8% (7/19) with severe pain (VRS=3, closed circle). B, Subanalysis revealed that the percentage of patients with high level of PF-NGF was significantly higher in 39 patients with mild to severe pain (VRS=1-3, closed triangle) than in 29 patients with no pain (VRS=0, closed diamond) (41.0% *vs* 17.2%; *, $p<0.05$). The horizontal broken line indicates 28 pg/mL as the cut-off value.

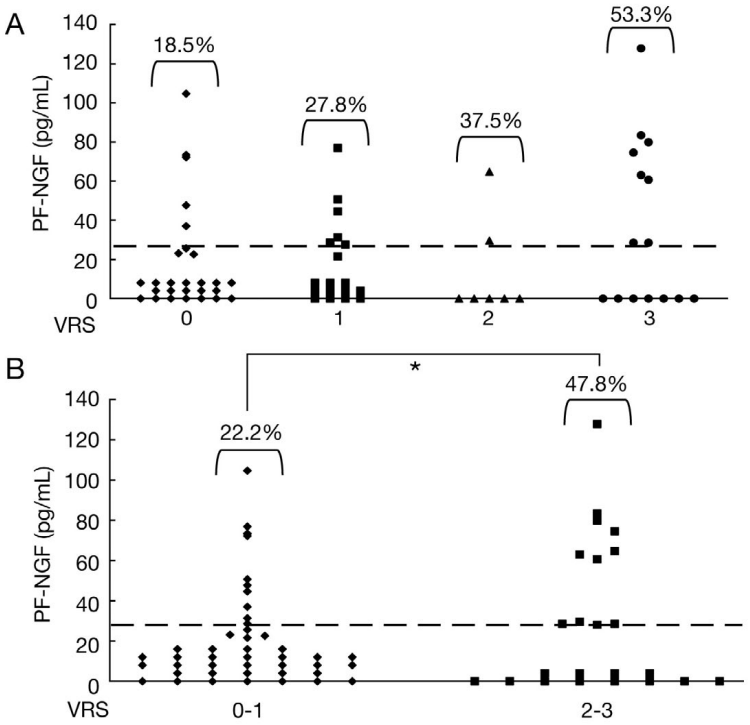


**Fig. 5** Relationship of the PF-NGF level and VRS for dyspareunia in patients with endometriosis
A, The PF-NGF concentration of each patient is dotted based on the VRS for dyspareunia. The horizontal broken line indicates 28 pg/mL as the cut-off value. Patients with high level of PF-NGF were found in 18.5 % (5/27) of patients with no pain (VRS=0, closed diamond), 27.8% (5/18) with mild pain (VRS=1, closed square), 37.5% (3/8) with moderate pain (VRS=2, closed triangle), and 53.3% (8/15) with severe pain (VRS=3, closed circle). B, Subanalysis revealed that the percentage of patients with high level of PF-NGF was significantly higher in 39 patients with moderate to severe pain (VRS=2-3, closed square) than in 29 patients with no or mild pain (VRS=0-1, closed diamond) (47.8% *vs* 22.2%; *, $p<0.05$). The horizontal broken line indicates 28 pg/mL as the cut-off value.

sion was up-regulated in artificially induced endometriotic lesions [22, 23]. We have shown for the first time that these lesions exhibit an abundance of NGF transcripts, which explains the elevated levels of its protein.

As NGF is transcriptionally up-regulated by an intracellular cAMP-dependent signaling pathway in the brain [24-28], it is highly likely that the prostaglandin E2-cAMP signaling pathway, which plays key roles in the growth and inflammation of endometriotic tissues [29], may participate in the induction of NGF mRNA expression in endometriosis. Alternatively, pro-inflammatory cytokines including interleukin-1β and tumor necrosis factor-α may stimulate NGF production [30]. These pro-inflammatory cytokines are locally produced and abundantly present in endometriotic lesions [29]. It is, therefore, conceivable that they may also contribute to the induction of NGF in those lesions.

NGF is a neurotrophin that regulates neuronal survival, growth, differentiation and synaptic plasticity through modifying the expression of a variety of genes including other neurotrophins, cytokines, growth factors, and nociceptors [9]. Thus, given the up-regulation of NGF in endometriotic lesions as presented here, it is highly likely that nerve fibers may proliferate in these lesions under the influence of NGF, ultimately leading to the development of pain. Recently, it has been reported that nerve fiber density and expression levels of NGF in deep infiltrating endometriosis are much higher than in superficial peritoneal endometriotic lesions [31]. Also, in rodent models of endometriosis, NGF expression is up-regulated with resulting development of numerous nerve fibers within the endometriotic lesions [22, 23, 32]. Notably, NGF can increase the number of sensory neurons and is selectively trophic for the small fiber sensory neurons and sympathetic ganglion neurons, which participate in mediating pain sensation [10, 33]. Based on these observations, the nerve fibers present in endometriotic lesions are most likely a mixture of sensory Ad, sensory C, cholinergic and adrenergic nerve fibers, as determined by the staining pattern

for the corresponding specific neuronal markers [31]. Taken together, among the neurotrophins, NGF, locally produced in the endometriotic lesions, may determine the density and type of nerve fibers produced within endometriotic lesions. In this way NGF may produce endometriosis-related pain such as dysmenorrhea and dyspareunia.

Besides its neurotrophic effects, NGF also promotes the sensitization and activation of nociceptor terminals in a transcription-independent way [9]. For instance, heat responses of nociceptive neurons in culture are increased acutely following NGF stimulation. *In vivo* animal studies have revealed that rats show behavioral signs of thermal and mechanical hyperalgesia within 15 min and 6 hr, respectively, after exogenous administration of NGF [34]. In humans, NGF administration has been reported to evoke systemic and injection site hyperalgesia [9]. We considered this observation in the context of the involvement of NGF in nociception and the rich innervation in peritoneal and deep infiltrating endometriosis [19, 20, 31]. This prompted us to hypothesize that the exposure of endometriotic lesions to PF-NGF may influence the sensitization and activation of nociceptors in these lesions, thereby promoting the generation of endometriosis-related pain. We therefore investigated the relationship between PF-NGF levels and the severity of dysmenorrhea and dyspareunia and found that elevated PF-NGF was more frequently observed in endometriosis patients with severe pain than in those with less severe pain. These results collectively suggest that NGF produced locally in peritoneal cavity may contribute to the generation and persistence of endometriosis-associated pelvic pain such as dysmenorrhea and dyspareunia.

It remains to be elucidated, however, whether the levels of PF-NGF (more than 20 -30 pg/mL) are high enough for NGF to exert its biological effects, in particular, sensitization of nociceptors. Sarchielli *et al*. have demonstrated that NGF levels (39.3 ± 5.9 pg/mL) in cerebrospinal fluid are significantly higher in patients with chronic daily headaches than in control subjects (11.3 ± 2.6 pg/mL). This suggests that NGF may play a role in the long-lasting sensitization underlying chronic headache [35]. In our study, the PF-NGF levels fell within the range in which sensitization and activation of nociceptors may have occurred on the sensory neurons present abundantly in the endometriotic lesions.

NGF has emerged as an enhancer of pain sensitization in a variety of diseases [9]. Therefore, targeting of NGF and its signaling pathway(s) may be a rational therapeutic strategy to relieve the NGF-induced acute and chronic pain. Indeed, a successful and effective blockade of NGF-related pain has been demonstrated in various animal models through inhibition of NGF function and its signaling pathway. This inhibition has been achieved with NGF-capturing antibodies, NGF receptor blockers and inhibitors of intracellular NGF signaling pathways [36]. In addition, treatment with a blocking antibody to NGF has been reported to effectively reduce pain and improve the quality of life in patients with tumor-induced bone cancer pain [18]. We and other investigators have demonstrated the involvement of NGF in endometriosis and its associated pain [19, 20, 31]; therefore, it is rational to treat endometriosis-associated pelvic pain by targeting NGF and its signaling pathway. In particular, a close association between PF-NGF levels and severity of endometriosis-related pain may substantiate a possible NGF-targeting therapy for patients with dysmenorrhea and/or dyspareunia together with elevated PF-NGF. Patients with NSAID-resistant pain may be most likely to benefit from NGF-targeting drugs either alone or in combination with NSAIDs.

As shown in Fig. 3, the percentage of patients with high PF-NGF was higher in the group with endometriosis than in the group without endometriosis (30.9% *vs* 25.0%), but the difference was not significant. Notably, the presence of a certain number of patients without endometriosis but with a high PF-NGF suggests that NGF may be produced not only from endometriotic lesions but also from other sources including peritoneal mast cells and macrophages. Indeed, NGF is a cytokine produced by a variety of cells including Schwann cells, keratinocytes, fibroblasts, T lymphocytes, mast cells, and macrophages [37, 38]. Additionally, among 12 patients who had no endometriosis but exhibited high PF-NGF levels (Fig. 3), only one (8.3%) and no patient complained of dysmenorrhea (VRS=2) and dyspareunia, respectively (data not shown), suggesting that not only NGF but other components of NGF signaling pathway such as NGF receptors may also play important roles in the generation and maintenance of pain. In support of this idea, Anaf *et al.* [19] have reported that NGF-specific tyrosine kinase receptor (TrkA) is strongly expressed in all the nerves that are surrounded and/or invaded by endometriosis or that are near endometriotic lesions. Furthermore, Tokushige *et al.* [20] have described that NGF receptor p75 (p75NTR)-im-

munoreactive nerve fibers are present near endometriotic glands in the peritoneal endometriotic lesions. Also, our preliminary RT-PCR analysis revealed that *p75NTR* expression was more abundant in endometriotic lesions than endometriosis-free eutopic endometrium (data not shown). It is, therefore, conceivable that the up-regulation of NGF receptors in endometriotic lesions may deteriorate endometriosis-associated dysmenorrhea and dyspareunia through enhancement of NGF action.

In conclusion, NGF locally induced and produced from endometriotic lesions may contribute to the generation and maintenance of acute and chronic pelvic pain, such as dysmenorrhea and dyspareunia. Although elucidation of the detailed molecular mechanism(s) underlying NGF-related pain awaits further studies, the results of this study support a role for targeting NGF in the treatment of endometriosis-associated pain.

## Acknowledgements

This work was supported, in part, by Grant-in-Aids from the Japan Society for the Promotion of Science (to T.K., T.M., H.A., and Y.Y.).

We are grateful to Dr. Akiko Yamasaki for sample collection and technical assistance and Ms. Rika Shibata for secretarial assistance.

## Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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