Endocrine Journal 2024, **71** (4), 395-401. doi:10.1507/endocrj.EJ23-0486

ORIGINAL

Expression of SMADs in orthotopic human endometrium, ovarian endometriosis, and endometriotic lesions in a murine model

Yuri Kadota, Takeshi Kato, Kana Kasai, Takako Kawakita, Misaki Murayama, Akari Shinya, Hikari Sasada, Sachiko Katayama, Mari Nii, Shota Yamamoto, Hiroki Noguchi, Kou Tamura, Hidenori Aoki, Miyu Taniguchi, Tomotaka Nakagawa, Takashi Kaji, Masato Nishimura, Riyo Kinouchi, Kanako Yoshida and Takeshi Iwasa

Department of Obstetrics and Gynecology, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima 770-8503, Japan

Abstract. Activin A promotes the development of endometriotic lesions in a murine model of endometriosis, and the immunohistochemical localization of phosphorylated suppressor of mothers against decapentaplegic homolog 2/3 (pSMAD2/3) complex in endometriotic lesions has been reported. Activin may therefore be involved in the development and proliferation of endometriotic cells *via* the SMAD signaling pathway. However, few detailed reports exist on SMAD7 expression in endometriosis. The purpose of this study was to investigate the expression of pSMAD2/3 or pSMAD3 and SMAD7 in the orthotopic human endometrium, ovarian endometriosis, and endometriotic lesions in a murine model and the effect of activin A on pSMAD2/3 and SMAD7 expression. We established an endometriosis murine model *via* the intraperitoneal administration of endometrial tissue and blood from donor mice. Activin A was intraperitoneally administered to the activin group. We immunohistochemically evaluated orthotopic endometria, ovarian endometriotic tissues, and endometriotic lesions in the murine model followed by western blotting. We found that pSMAD3 and SMAD7 were expressed in ovarian endometriosis and orthotopic endometria from patients with and without endometriosis. In the murine model, endometriotic lesions expressed pSMAD2/3 and SMAD7 in the activin and control groups, and higher SMAD7 expression was found in the activin group. To the best of our knowledge, this study is the first to show that SMAD7 expression is upregulated in endometriosis. In conclusion, these results suggest that activin A activates the SMAD signaling pathway and promotes the development of endometriotic lesions, thus identifying SMAD7 as a potential therapeutic target for endometriosis.

Key words: Endometriosis, Suppressor of mothers against decapentaplegic (SMAD), Suppressor of mothers against decapentaplegic 7 (SMAD7), Activin A

ENDOMETRIOSIS involves the extrauterine development of normal endometrium-like tissues and is a common gynecological disorder that affects 6–10% of women of reproductive age. Endometriosis causes dysmenorrhea and infertility and significantly impairs quality of life [1]. Although the pathogenesis of endometriosis is still unclear, the retrograde menstruation theory, which purports that the orthotopic endometrium penetrates the peritoneal cavity during menstruation and implants into the pelvic peritoneum, is widely accepted [2-4].

Activin is a double-stranded glycoprotein that belongs to the transforming growth factor beta (TGFB) superfamily and promotes the secretion of FSH from the pituitary gland [5]. Additionally, activin acts as a local regulator in a paracrine or autocrine manner to exert various physiological effects on embryonic development [6], neuroprotection [7], cell apoptosis [8, 9], and fibrosis [10]. The major activin transduction pathway is the suppressor of mothers against decapentaplegic (SMAD) signaling pathway. Activin first binds to the type 2 receptors activin A receptor type 2A (ACVR2A) and type 2B (ACVR2B) and then to the type 1 receptors ACVR1A and ACVR1B, also known as ALK4, to form a complex. At this point, the type 1 receptor is phosphorylated, and

Submitted Sep. 15, 2023; Accepted Dec. 27, 2023 as EJ23-0486
Released online in J-STAGE as advance publication Feb. 28, 2024
Correspondence to: Yuri Kadota, Department of Obstetrics and Gynecology, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto-Cho, Tokushima 770-8503, Japan.
E-mail: kadota.yuri@tokushima-u.ac.jp

this phosphorylates the receptor-regulated SMADs (R-SMADs) SMAD2 and SMAD3 and forms a complex with SMAD4, which then enters the nucleus. In the nucleus, SMAD complexes bind to the transcription factors of various target genes and regulate their expression [11]. The inhibitory SMAD (I-SMAD) SMAD7 inhibits the activation of R-SMADs by binding to type 1 receptors activated by type 2 receptors, thereby preventing the activation of the SMAD signaling pathway. SMAD7 is also induced by activin and inhibits its signaling *via* a negative feedback mechanism [12-14].

Patients with endometriosis have been reported to exhibit higher concentrations of activin in the peritoneal fluid than in women without endometriosis [15]. Patients with endometriosis also have higher serum activin concentrations than those of healthy women [16]. We have previously reported that the intraperitoneal administration of activin A promotes the development of endometriotic lesions in a murine model and showed the immunohistochemical localization of pSMAD2/3 in endometriotic lesions [17]. Furthermore, Mabuchi *et al.* [18] reported the immunohistological localization of SMAD2, SMAD3, and SMAD4 in orthotopic endometrial tissue and ovarian endometriotic lesions. Therefore, activin is likely involved in the development and proliferation of endometriotic cells *via* the SMAD signaling pathway. However, the expression of SMAD7 in endometriotic lesions has not been investigated in detail. Therefore, we investigated the expression of pSMAD3 or pSMAD2/3 and SMAD7 in orthotopic human endometria and ovarian endometriosis and endometriotic lesions in a murine model. Additionally, we evaluated the effect of activin A on the expression of pSMAD2/3 and SMAD7.

Materials and Methods

Patient selection, characterization, and sample preparation

This study was approved by the ethics committee of the Tokushima University Hospital (No. 3836).

Ovarian endometriosis and uterine-endometrium samples were obtained from 10 patients admitted to the Tokushima University Hospital between 2015 and 2020. The diagnoses were established *via* laparoscopy and subsequent histological examination of the endometriotic lesions. Normal uterine-endometrium tissues were obtained from five patients without endometriosis who underwent total hysterectomy for uterine cervical intraepithelial neoplasia. Informed consent was obtained *via* an opt-out format on the Institute of Clinical Review Board website. Endometrial and ovarian endometriotic tissues were fixed in 4% paraformaldehyde and embedded in paraffin.

Murine model

Experimental mice

Female C57BL/6J mice, aged 7 weeks, were purchased from Charles River Laboratories Japan (Yokohama, Japan) and CLEA Japan (Tokyo, Japan). The mice had unrestricted access to food and water and were exposed to alternating light–dark cycles (12 h each) in a controlled environment. Invasive procedures were performed under sevoflurane anesthesia. The surgery was performed under sterile conditions [15]. The experimental protocol involving animals was approved by the Institutional Animal Care and Use Committee of the University of Tokushima (No. T2020-53).

Induction of endometriotic lesions and activin treatment

Donor mice were ovariectomized and subcutaneously injected with estrogen (2.0 μg/mouse/day) (β-estradiol; Sigma-Aldrich, St. Louis, MO, USA) in peanut oil (0.1 mL) daily. The donor mice were euthanized 7 days after the ovariectomy. Their uteri were removed and placed in 0.2 mL of phosphate-buffered saline (PBS). The endometrium was gently peeled from the uterine muscle and cut into small pieces (approximately 1 mm in diameter) with small scissors. Recipient mice were intraperitoneally injected with endometrial fragments in PBS and 0.1 mL donor blood. The treatment group (n = 8) received intraperitoneal injections of activin A (400 ng/mouse; R&D Systems, Minneapolis, MN, USA), and the control group (n = 8) was injected with PBS daily for five days [17].

Evaluation of murine endometriotic lesions

The recipient mice were euthanized 5 days after the surgical procedure. The peritoneal cavity of each mouse was examined. Endometriotic lesions were measured, and sizes were reported as the maximum diameter and total area of the lesions for each mouse. The lesions were excised, fixed in 4% paraformaldehyde, and embedded in paraffin [17].

Immunohistochemical detection of estrogen receptor 1, SMAD2/3, and SMAD7

Paraffin-embedded tissues were cut into 5-μm sections, deparaffinized, and rehydrated using xylene and a graded series of alcohol solutions. Antigen unmasking was performed using Antigen Unmasking Solution (Vector Laboratories, Newark, CA, USA) in a pressure cooker for 5 min. Endogenous peroxidase activity was quenched using 3% H_2O_2 for 15 min. Nonspecific binding was blocked using 2.5% normal horse serum for 20 min. Estrogen receptor 1 (ESR1) (1:2000) (ab32063; Abcam, Cambridge, UK), SMAD7 (1:200) (ARG42203; arigo Biolaboratories, Hsinchu, Taiwan), and SMAD3 (phosphorylated S423 + S425) antibodies (1:200) (ab52903; Abcam, Cambridge, UK) were used as

primary antibodies. The sections were incubated with primary antibodies for 30 min (ESR and SMAD7) or overnight (SMAD3) at 24°C. The sections were then incubated with ImmPRESS Anti-Rabbit IgG (Vector Laboratories) for 30 min. Antigens were detected using diaminobenzidine staining (2.5 min), and sections were counterstained with hematoxylin QS (Vector Laboratories) for 30 s. Finally, the sections were dehydrated and mounted. Negative control slides were prepared by replacing the primary antibody with PBS, as previously described [17].

Western blotting

Tissues were lysed in Tissue Protein Extraction Reagent (T-PER, Thermo Fisher Scientific, Waltham, Tokyo, Japan) with Halt™ Protease Inhibitor Cocktail and Halt™ Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific). The tissue was homogenized and centrifuged and the supernatant was collected. Protein concentration was measured using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). A total of 20 μg of protein samples were separated *via* gel electrophoresis on 10% Mini-PROTEAN TGX Gels (Bio-Rad Laboratories, Inc., Tokyo, Japan) and transferred onto a polyvinylidene difluoride membrane. The membranes were blocked with 3% bovine serum albumin in Tris-buffered saline with 0.1% Tween-20 at 24°C for one hour. Next, membranes were incubated with primary antibodies Anti-SMAD2+3 phospho (Ser465/467)/phosphor (Ser423/425) (1:250) (ARG40897; arigo Biolaboratories), Human/Mouse/Rat SMAD7 (4 μg/mL) (338-AC; R&D Systems), and β-Actin (8H10D10) Mouse mAb (1:1000)(#3700; Cell Signaling Technology, Beverly, MA, USA) overnight at 4°C. After washing, the membrane was treated with secondary antibodies (1:2000) for one hour at 24°C. Proteins were detected using the Amersham ECL Prime Western Blotting Detection Reagent (GE HealthCare, Chicago, IL, USA) and visualized using an image analyzer (Amersham Imager 600; GE HealthCare). Densitometry was performed to analyze the differences in chemiluminescence intensity using ImageJ software version 1.53 (National Institutes of Health, Bethesda, MA, USA).

Statistical analyses

To compare groups, the Mann–Whitney U test was used for non-normally distributed data, and Student's *t* test was used for normally distributed data. $p < 0.05$ was considered statistically significant. All statistical analyses were performed using R version 4.0.1 [19].

Results

Patient's baseline characteristics were shown in Table 1. Women with endometriosis had an age range of 40 to 50 years (mean ± standard deviation: 46.3 ± 3.3). Diagnosis was confirmed *via* laparoscopy and histological examination of the endometriotic lesions. Women without endometriosis were between 36 and 47 years of age (mean ± standard deviation: 42.0 ± 3.5) (Table 1).

Table 1 Baseline characteristics

		patients with endometriosis ($n = 10$)	women without endometriosis ($n = 5$)
age (y)		46.3 ± 3.3	42.0 ± 3.5
Body mass index		22.1 ± 3.1	22.1 ± 2.7
Parity	0	5	1
	≥1	5	4
diagnosis		endometriosis	Uterine cervical neoplasia

Fig. 1 shows the immunolocalization of pSMAD3 and SMAD7 in ovarian endometriosis tissues and the orthotopic endometria of patients with and without endometriosis. In ovarian endometriotic tissues and orthotopic endometria of patients with and without endometriosis, we observed positive immunoreactive staining for pSMAD3 and SMAD7 in the cytoplasm and nucleus (Fig. 1).

In the control group of the murine model, endometriotic lesions in the abdominal cavities of six mice were found, whereas lesions were found in all mice in the activin group (Fig. 2A, B). Most lesions were found in the peritoneal incision or mesentery. ER1 was expressed in epithelial and stromal cells of lesions (Fig. 2C). The maximum diameter of lesions was significantly larger in the activin group (4.0 ± 1.7 mm) than in the control group (1.25 ± 1.3 mm, $p < 0.01$) (Fig. 3A). The total lesion area in the activin group (19.8 ± 16.0 mm^2) was significantly larger than that in the control group (3.6 ± 4.1 mm^2, $p < 0.01$) (Fig. 3B).

Fig. 4 depicts the immunohistochemical localization of pSMAD2/3 and SMAD7 in the murine endometriotic lesions. In both the control and activin-treated groups, we observed positive pSMAD2/3 and SMAD7 immunoreactive staining in the cytoplasm and nuclei (Fig. 4).

Western blot analysis indicated increased SMAD7 expression in the activin group compared with that in the control group. However, no significant difference in pSMAD2/3 expression was found (Fig. 5).

Discussion

The retrograde menstruation theory is commonly used to explain the development of endometriosis [2-4].

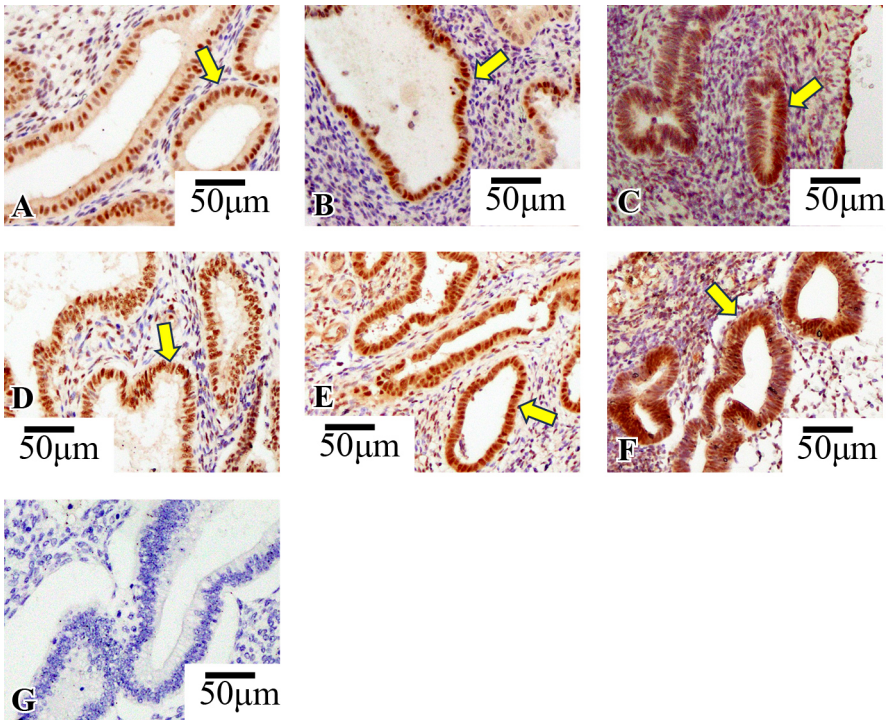

Fig. 1 Immunohistochemical localization of pSMAD2/3 and SMAD7 in ovarian endometriotic tissues and the normal endometrium. Brown staining indicates immunoreactivity

(A–C) pSMAD2/3. (D–F) SMAD7. (G) Negative control. Normal endometria of women without endometriosis are depicted in the left panels (A, D, and G). Normal endometria of patients with endometriosis are shown in the middle panels (B and E). Ovarian endometriosis is shown in the right panels (C and F). Original magnification: ×100

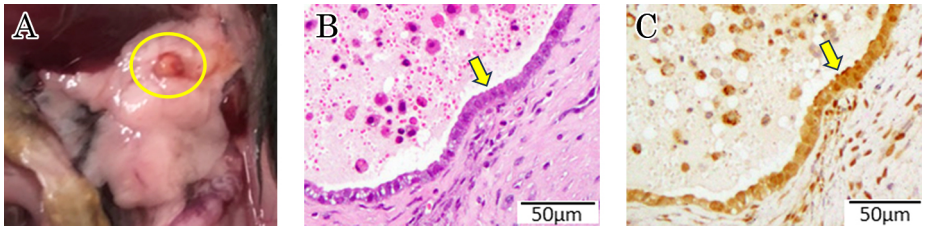

Fig. 2 Endometriotic lesions

(A) Endometriotic lesion. (B) Hematoxylin–eosin-stained lesion. (C) Immunohistochemical detection of estrogen receptor 1. Original magnification: ×100

However, although menstrual reflux occurs in 75–90% of women, endometriosis occurs in only some women [4]. Therefore, both menstrual blood reflux and intra-abdominal and endometrial differences may cause endometriosis. Endometriotic SMAD7 expression has not been widely studied. Hence, we investigated the expression of pSMAD3, SMAD2/3, and SMAD7 in orthotopic human endometria, ovarian endometriosis, and endometriotic lesions in a murine model created by transplanting endometrial tissue and blood into the abdominal cavity to mimic menstrual reflux. To the best of our knowledge, the present study is the first to show that SMAD7 expression is increased in endometriosis.

Activin was discovered in the follicular fluid as a factor that promotes FSH secretion in the pituitary gland and performs numerous physiological activities. The SMAD pathway is the major pathway for the nuclear transmission of activin. In mammals, eight types of SMAD are described and classified into three types based on their actions. R-SMAD is specifically activated by TGFB family receptors, such as activin, common SMADs, which bind to and act on R-SMADs, and I-SMADs, which have inhibitory effects. R-SMADs comprise SMAD1, SMAD2, SMAD3, SMAD5, and SMAD8. High structural homology is observed between SMAD2 and SMAD3, and between SMAD1, SMAD5,

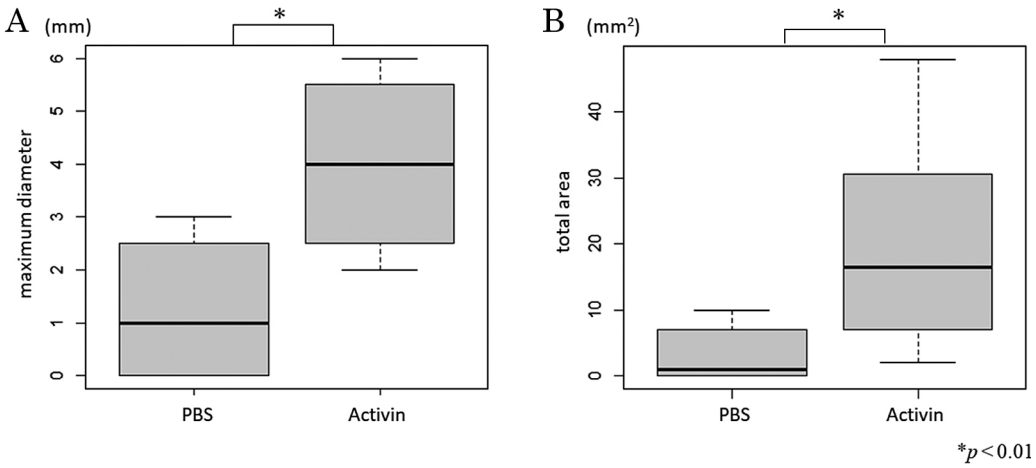

Fig. 3 Maximum diameter (A) and total area (B) of murine model endometriotic lesions

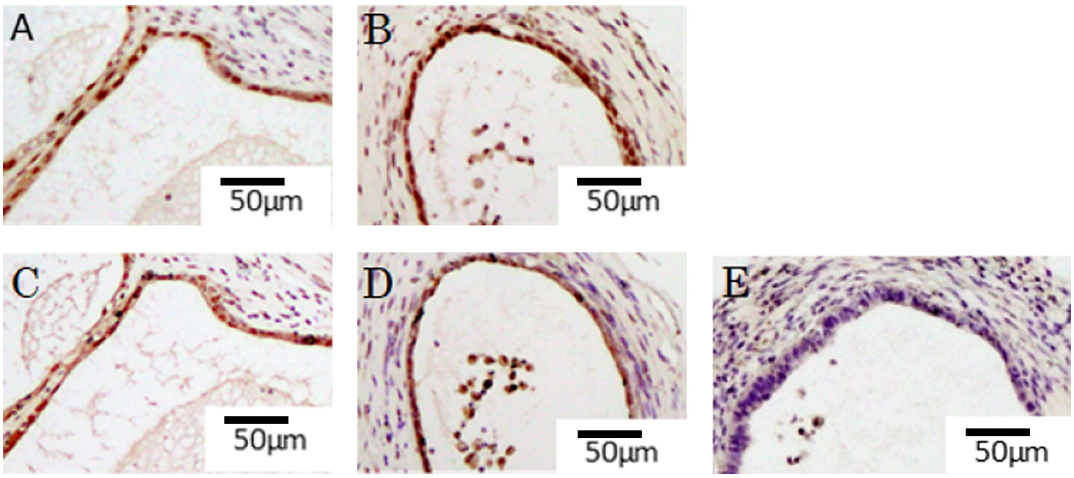

Fig. 4 Immunohistochemical localization of pSMAD2/3 and SMAD7 in murine endometriotic lesions. Brown staining indicates immunoreactivity

(A, B), pSMAD2/3. (C, D) SMAD7. (E) Negative control. The control group is represented in the left panels (A, C). The activin group is represented in the right panels (B, D). Original magnification: ×100

and SMAD8, with SMAD2 and SMAD3 transducing TGFB and activin signals, respectively. SMAD6 and SMAD7, which are I-SMADs, stably bind to type 1 receptors activated by type 2 receptors and suppress the activation of R-SMADs. SMAD6 has a stronger effect on bone morphogenetic proteins and a weaker inhibitory effect on activin and TGFB [11-14].

In this study, immunohistochemical staining revealed that pSMAD2/3 and SMAD7 were expressed in both the orthotopic endometria and ovarian endometriotic tissues of patients with and without endometriosis. We found that the intraperitoneal administration of activin promoted lesion development, which confirms previous reports [17], and that pSMAD2/3 and SMAD7 were expressed in endometriotic lesions in the control and activin groups in the murine model. These results suggest that activin promotes endometriosis *via* the SMAD signaling pathway.

Furthermore, in this study, Western blot analysis of murine endometriosis lesions, depicted a stronger expression of Smad7 in the activin group than in the PBS group. Although no statistically significant differences were observed for SMAD2/3, the results suggested a similar trend to SMAD7, suggesting that the activin group compared to the PBS group. Normally, the addition of activin causes phosphorylation of Smad2/3 and translocation of pSMAD2/3 into the nucleus. Subsequent negative feedback induces inhibitory SMAD7, which is presumed to repress Smad2/3. However, elevated levels of activin have been reported in peritoneal fluid of women with endometriosis compared to those without endometriosis [15]. Thus, it is plausible that under

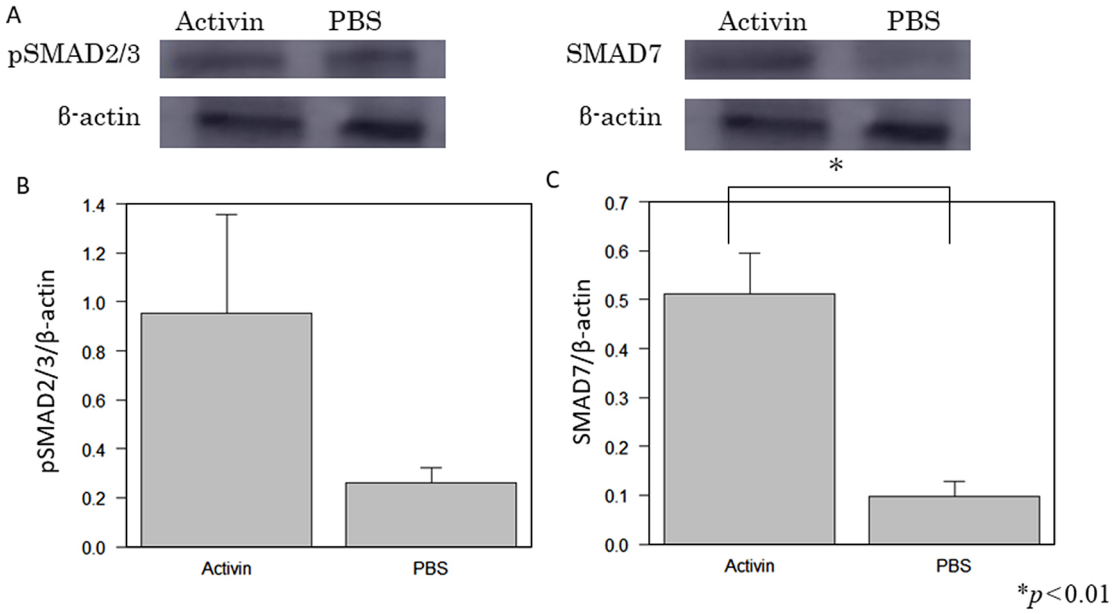

Fig. 5 pSMAD2/3 and SMAD7 expression levels in a murine model of endometriosis
(A) Representative western blots of pSMAD2/3 and SMAD7. Quantified expression levels of pSMAD2/3 (B) and SMAD7 (C).

conditions of high activin levels, the SMAD pathway is constantly activated and the expression of both pSMAD2/3 and SMAD7 is elevated due to constant exposure to activin stimulation. This sustained activation of the SMAD pathway was thought to be involved in the development of endometriosis.

In scleroderma, a chronic inflammatory disease, which is considered similar to endometriosis, an upregulated expression of Smad7 molecules in fibroblasts from patients with scleroderma has been reported. Besides, Smad2/3 phosphorylation is enhanced, coupled with an impaired inhibitory effect of Smad7 on TGFB signaling, a pathway transmitted through SMAD signaling, similar to activin [20]. It is possible that in our study, dysfunction of SMAD7 in endometriosis lesions impaired its inhibitory effect on the SMAD pathway, resulting in increased SMAD7 expression. Notably, in Crohn's disease and ulcerative colitis, SMAD7 expression is also increased in mucosal infiltrating T cells, and it had been reported that inflammatory cytokines were reduced by SMAD7 inhibitors [21]. Oral administration of a SMAD7 inhibitor in a murine model of colitis has been reported to suppress colitis and intestinal fibrosis [22]. These reports underscore the involvement of the SMAD pathway in the pathogenesis of inflammatory diseases and suggest the importance of SMAD7 in inflammation and fibrosis.

The involvement of the SMAD signaling pathway in endometriosis has also been reported. Mabuchi *et al.* [18] reported the expression of SMAD2, 3, and 4 in human endometriotic lesions and orthotopic endometria. Collectively, these reports suggest that, as in other chronic inflammatory diseases, the SMAD signaling pathway is implicated in endometriosis, with a significant role of the SMAD7 upregulation.

Recently, in human endometriotic cells, TGFB1 has been reported to suppress apoptotic cell death *via* a cascade involving TGFB-activated kinase 1, nuclear factor kappa light-chain enhancer of activated B cells p65, and SMAD7 (TAK1–NFKB p65–SMAD7) and which promotes endometriotic cell proliferation [23]. Additionally, it was reported that TGFB1 suppresses cell proliferation, increases apoptosis, and promotes tumor progression *via* the TAK1–NFKB p65–SMAD7 pathway in head and neck cancer [24]. In the present study, intraperitoneal administration of activin in an endometriosis murine model may have increased SMAD7 expression and promoted lesion formation *via* the TAK1–NFKB p65–SMAD7 pathway; however, this mechanism of pathogenesis requires confirmation in future studies.

In conclusion, we demonstrated that pSMAD2/3 and SMAD7 were expressed in the orthotopic endometria in patients with and without endometriosis, as well as in ovarian endometriotic tissues. In addition, pSMAD2/3 and SMAD7 were expressed in endometriotic lesions in a murine model, and SMAD7 expression was enhanced following an intraperitoneal administration of activin. This suggests that activin A activates the SMAD signaling pathway and promotes the development of endometriotic lesions. Therefore, activin may promote endometriosis, and SMAD7 may be a new therapeutic target for endometriosis.

Acknowledgments

This study was supported by the Support Center for Advanced Medical Sciences, Tokushima University Graduate School of Biomedical Sciences. We would like to thank Editage (www.editage.jp) for English language editing.

Disclosure

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

References

1. Eskenazi B, Warner ML (1997) Epidemiology of endometriosis. *Obstet Gynecol Clin North Am* 24: 235–258.
2. Sampson JA (1927) Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol* 14: 422–469.
3. Bartosik D, Jacobs SL, Kelly LJ (1986) Endometrial tissue in peritoneal fluid. *Fertil Steril* 46: 796–800.
4. D'Hooghe TM, Debrock S (2002) Endometriosis, retrograde menstruation and peritoneal inflammation in women and in baboons. *Hum Reprod Update* 8: 84–88.
5. Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, *et al.* (1986) Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin. *Nature* 321: 779–782.
6. Hemmati-Brivanlou A, Melton DA (1992) A truncated activin receptor inhibits mesoderm induction and formation of axial structures in Xenopus embryos. *Nature* 359: 609–614.
7. Tretter YP, Munz B, Hübner G, ten Bruggencate G, Werner S, *et al.* (1996) Strong induction of activin expression after hippocampal lesion. *NeuroReport* 7: 1819–1823.
8. Chen W, Woodruff TK, Mayo KE (2000) Activin A-induced HepG2 liver cell apoptosis: involvement of activin receptors and SMAD proteins. *Endocrinology* 141: 1263–1272.
9. Carey JL, Sasur LM, Kawakubo H, Gupta V, Christian B, *et al.* (2004) Mutually antagonistic effects of androgen and activin in the regulation of prostate cancer cell growth. *Mol Endocrinol* 18: 696–707.
10. Werner S, Alzheimer C (2006) Roles of activin in tissue repair, fibrosis, and inflammatory disease. *Cytokine Growth Factor Rev* 17: 157–171.
11. Massagué J (1998) TGF-β signal transduction. *Annu Rev Biochem* 67: 753–791.
12. Hayashi H, Abdollah S, Qiu Y, Cai J, Xu YY, *et al.* (1997) The MAD-related protein SMAD7 associates with the TGFβ receptor and functions as an antagonist of TGFβ signaling. *Cell* 89: 1165–1173.
13. Imamura T, Takase M, Nishihara A, Oeda E, Hanai J, *et al.* (1997) SMAD6 inhibits signalling by the TGF-beta superfamily. *Nature* 389: 622–626.
14. Nakao A, Afrakhte M, Morén A, Nakayama T, Christian JL, *et al.* (1997) Identification of Smad7, a TGF-β-inducible antagonist of TGF-β-signalling. *Nature* 389: 631–635.
15. Hou Z, Sun L, Gao L, Liao L, Mao Y, *et al.* (2009) Cytokine array analysis of peritoneal fluid between women with endometriosis of different stages and those without endometriosis. *Biomarkers* 14: 604–618.
16. Reis FM, Luisi S, Abrão MS, Rocha AL, Viganò P, *et al.* (2012) Diagnostic value of serum activin A and follistatin levels in women with peritoneal, ovarian and deep infiltrating endometriosis. *Hum. Reprod* 27: 1445–1450.
17. Kasai K, Kato T, Kadota Y, Erdenebayar O, Keyama K, *et al.* (2019) Intraperitoneal administration of activin A promotes development of endometriotic lesions in a mouse model of endometriosis. *J Med Invest* 66: 123–127.
18. Mabuchi Y, Yamoto M, Minami S, Umesaki N (2010) Immunohistochemical localization of inhibin and activin subunits, activin receptors and Smads in ovarian endometriosis. *Int J Mol Med* 25: 17–23.
19. R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/ accessed on March 8, 2023.
20. Asano Y, Ihn H, Yamane K, Kubo M, Tamaki K (2004) Impaired Smad7-Smurf-mediated negative regulation of TGF-β signaling in scleroderma fibroblasts. *J Clin Invest* 113: 253–264.
21. Monteleone G, Kumberova A, Croft NM, McKenzie C, Steer HW, *et al.* (2001) Blocking Smad7 restores TGF-beta 1 signaling in chronic inflammatory bowel disease. *J Clin Invest* 108: 601–609.
22. Izzo R, Bevivino G, De Simone V, Sedda S, Monteleone I, *et al.* (2018) Knockdown of Smad7 with a specific antisense oligonucleotide attenuates colitis and colitis-driven colonic fibrosis in mice. *Inflamm Bowel Dis* 24: 1213–1224.
23. Kaushal JB, Shukla V, Sankhwar P, Jha RK, Dwivedi A (2021) Targeted inhibition of TAK1 abrogates TGFβ1 non-canonical signaling axis NFκ/SMAD7 inhibiting human endometriotic cells proliferation and inducing cell death involving autophagy. *Cytokine* 148: 155700.
24. Freudlsperger C, Bian Y, Contag Wise S, Burnett J, Coupar J, *et al.* (2013) TGF-β and NF-κB signal pathway cross-talk is mediated through TAK1 and SMAD7 in a subset of head and neck cancers. *Oncogene* 32: 1549–1559.