NAG-1 expression and apoptosis induction in endometrial stromal cells of patients with endometriosis by histone deacetylase inhibitor trichostatin A

Seok Kyo Seo

Department of Medicine

The Graduate School, Yonsei University

NAG-1 expression and apoptosis induction in endometrial stromal cells of patients with endometriosis by histone deacetylase inhibitor trichostatin A

Directed by Professor Byung Seok Lee

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Seok Kyo Seo

December 2015

This certifies that the Doctoral Dissertation of Seok Kyo Seo is approved.

Thesis Supervisor: Byung Seok Lee

Thesis Committee Member#1: Tak Kim

Thesis Committee Member#2: Nam Hoon Cho

Thesis Committee Member#3: Chae-Ok Yun

Thesis Committee Member#4: Kyung Joo Hwang

The Graduate School
Yonsei University

December 2015

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to professor Byung-Seok Lee, who has helped me complete this thesis with his steady support and advice, despite his busy schedule and work. Also, my gratitude extends to professor Tak Kim, Nam-Hoon Cho, Chae-Ok Yoon, and Kyung-Joo Hwang, who have contributed to this thesis with sincere interest and meticulous guidance.

I would also like to express my gratitude to my mother and other family members, who always lift me up support me with tremendous love and encouragement.

Finally, I dedicate this thesis to my loving wife Hye-Jung Joo, my life companion, and my two daughters Jae-Hyun and Ju-Hyun, who are the meaning of my life.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

NAG-1 expression and apoptosis induction in endometrial stromal cells of patients with endometriosis by histone deacetylase inhibitor trichostatin A

Seok Kyo Seo

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Byung Seok Lee)

Objective: The present study investigated the effects of trichostatin A (TSA) on non-steroidal anti-inflammatory drug-activated gene-1 (NAG-1) expression and apoptosis in human endometrial stromal cells (HESCs) and assessed whether these effects are enhanced in the presence of 5-aza-2'-deoxycytidine (5-aza-dC).

Methods: Eutopic endometrial tissue samples were obtained from 10 patients with fibroids who underwent hysterectomy. Ectopic endometrial tissues were obtained from 15 patients with endometriotic cysts who underwent cystectomy. HESCs were isolated and cultured with different concentrations of TSA. Real-time PCR and western blotting were used to evaluate NAG-1 mRNA and protein levels, respectively. Apoptosis was assessed by flow cytometry.

Results: TSA induced NAG-1 expression and apoptosis in HESCs. Gene knockdown experiments using small interfering RNA confirmed an association between NAG-1 expression and TSA-induced apoptosis. We did not observe synergy in the apoptosis-inducing effects of TSA and 5-aza-dC in HESCs.

Conclusion: TSA induced apoptosis in HESCs via induction of NAG-1 expression. These results suggest that TSA—and possibly other HDACIs or agents that induce NAG-1 expression—can be effective for treating endometriosis.

Key words: endometriosis, epigenetics, trichostatin A, NAG-1

NAG-1 expression and apoptosis induction in endometrial stromal cells of patients with endometriosis by histone deacetylase inhibitor trichostatin A

Seok Kyo Seo

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Byung Seok Lee)

I. INTRODUCTION

Endometriosis, in which endometrial glands and stroma are located outside the uterus, is a benign disease that is associated with dysmenorrhea, pelvic pain, low fertility, and reduced quality of life. The estimated prevalence of endometriosis is 10% among women of reproductive age and 20%–50% of infertile women.[1,2] Despite being a common and critical health problem in women, its pathogenesis and optimal course of management have not been clearly established.

Epigenetic modifications play an important role in regulating patterns of gene expression and are implicated in many cellular processes including development, differentiation, and tumorigenesis. The dysregulation of epigenetic processes has been linked to a variety of human diseases. Accordingly, epigenetic modifiers such as DNA demethylating agents and histone deacetylase inhibitors (HDACIs) have been approved by the U.S. Food and Drug Administration (FDA), with many similar drugs currently under development.

There is increasing evidence that endometriosis is an epigenetic disease that can potentially be treated with epigenetic modifiers such as trichostatin A (TSA), an HDACI.[3] TSA suppressed the proliferation and invasiveness of endometrial cells[4,5] while reducing endometrial implants in mice.[6] However, the mechanistic basis for

the effects of TSA on endometrial cells has yet to be elucidated.

Non-steroidal anti-inflammatory drug-activated gene (NAG)-1 is a member of the transforming growth factor (TGF)-β superfamily that is associated with cell growth, differentiation, and apoptosis.[7,8] Our previous study showed that NAG-1 expression was decreased in the endometrium of endometriosis patients, and that upregulation of NAG-1 expression was associated with increased apoptosis in human endometrial stromal cells (HESCs).[9] These results suggest that NAG-1 activation is a promising therapeutic approach in the treatment of endometriosis. NAG-1 expression can be regulated by various cyclooxygenase inhibitors,[9,10] peroxisome proliferator-activated receptor agonists,[11] and anti-neoplastic agents.[12]

A previous study found a link between NAG-1 and TSA-induced apoptosis in a human glioblastoma cell line.[13] However, whether this relationship exists in endometrial cells is not known. It is also unclear whether TSA can act synergistically with 5-aza-2'-deoxycytidine (5-aza-dC), a DNA demethylating agent, in endometrial cells. The present study investigated the effects of TSA on NAG-1 expression and apoptosis in HESCs and assessed whether these effects are enhanced in the presence of 5-aza-dC.

II. MATERIALS AND METHODS

Ethical approval

Written informed consent was obtained from all participants before surgery in accordance with the study protocol, which was approved by the Institutional Review Board of Gangnam Severance Hospital.

HESC isolation and culture

Eutopic endometrial tissue was obtained from 10 patients with fibroids who underwent hysterectomy. Ectopic endometrial tissue was obtained from 15 patients with endometrioma who underwent cystectomy. Isolated HESCs were cultured as previously described.[9] Briefly, endometrial tissue samples were minced into small pieces. Eutopic endometrial tissue was incubated in 5 ml of 0.25% trypsin-EDTA solution for 20 min and ectopic endometrial tissue was incubated with 2 mg/ml collagenase for 2 h in Dulbecco's Modified Eagle's Medium (DMEM)/F12 (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS; Gibco-BRL) at 37°C with constant agitation. Dispersed cells were centrifuged at 2000 rpm for 5 min, and the cell pellet was re-suspended in DMEM/F12 supplemented with 10% FBS. The stromal-enriched fraction was cultured in 75-ml culture flasks at 37°C in a humidified atmosphere of 95% air and 5% CO2. The purity of HESCs was assessed by immunohistochemistry using antibodies against vimentin, cytokeratin, and cluster of differentiation (CD)10.

Cell viability assay

Cell viability was assessed using the 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay. HESCs (1×10^4) were seeded in 96-well plates; after incubation for 24 h in DMEM/F12 with 10% FBS, cells

were treated with TSA and/or 5-aza-dC in DMEM/F12 with 2% FBS. A 100-μl volume of MTT solution (Sigma, St. Louis, MO, USA) was added to each well, followed by incubation at 37°C for 4 h in a 5% CO2 incubator. Culture supernatant was harvested for analysis with the enzyme-linked immunosorbent assay and by western blotting. The medium was replaced with 500 μl dimethyl sulfoxide (DMSO; Sigma) and cells were incubated for 10 min on a shaker. Optical density was measured at 560 nm on a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Analysis of HESC apoptosis by flow cytometry

HESCs (1×10^6) were seeded in 60-mm culture dishes and incubated for 48 h in DMEM/F12 containing 10% FBS. The medium was replaced with one containing 2% FBS, and cells were treated with TSA and/or 5-aza-dC, then stained with 5 μl fluorescein isothiocyanate/annexin V and propidium iodide (PI) according to the manufacturer's protocol. Apoptotic cells were identified by flow cytometry (FC 500; Beckman Coulter, Fullerton, CA, USA) and data were analyzed using WinMDI v.2.9 software (The Scripps Research Institute, San Diego, CA, USA).

RNA extraction and real-time (RT-)PCR

HESCs (5×10^5 per well) were seeded in 6-well plates in DMEM/F12 with 10% FBS. After 48 h, cells were washed and cultured in DMEM/F12 with 2% FBS and treated with TSA. Total RNA was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA, USA) and 2 μg were reverse transcribed into cDNA using the SuperScript III first-strand synthesis kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. NAG-1 mRNA levels were quantified by SYBR Green RT-PCR on an ABI PRISM 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a housekeeping gene, was used as an internal control. The forward and reverse primer sequences were designed as follows: NAG-1,

5'-CCCGAGAGATACGCAGGT-3' and 5'-GAAGATTCGAACACCGACCTC-3'; and GAPDH, 5'-CTTGAATCCCGAATGGAAAGGG-3' and 5'-CCTTCCCAAATAGAACCCCCA-3'. PCR amplification was carried out with 2 μl cDNA, 1 μl each primer, and 10 μl SYBR Green master mix (Applied Biosystems). Thermal cycling conditions were 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, 1 min at 60°C, and 15 s at 95°C. Relative NAG-1 mRNA levels were determined with the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

Cells were lysed in radioimmunoprecipitation assay buffer (iNtRON Biotechnology, Sungnam, Korea) containing a protease inhibitor cocktail (Cell Signaling Technology, Beverly, MA, USA). Lysates were mixed and divided into 40-μl aliquots, which were centrifuged at 13,000 rpm at 4°C for 30 min. Protein levels in the supernatant were quantified using a bicinchoninic protein assay kit (Thermo Scientific, Hudson, NH, USA); 30 μg of protein were boiled in sample buffer and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on 8% gels and transferred to a polyvinylidene difluoride membrane (Millipore, Eschborn, Germany). After blocking with bovine serum albumin at room temperature for 1 h, membranes were incubated with a primary antibody against NAG-1 (goat polyclonal anti-GDF15, 1:1000; Abcam, Cambridge, MA, USA) overnight at 4°C, followed by incubation with horseradish peroxidase-conjugated anti-goat secondary antibody (1:3000; Jackson, West Grove, PA, USA) for 1 h at room temperature. Protein bands were detected by enhanced chemiluminescence (Advansta, San Francisco, CA, USA) and were quantified by densitometry using Image J software (National Institutes of Health, Bethesda, MD, USA).

Short interfering (si)RNA transfection

NAG-1 siRNA was purchased from Bioneer Dharmacon (Lafayette, CO, USA). HESCs were transfected with siRNA oligonucleotides using Lipofectamine

RNAiMAX (Invitrogen) according to the manufacturer's recommendations. After 4 h, cells were washed and incubated overnight in complete medium. The following day, cells were treated with TSA or vehicle for 24 h. The effect of NAG-1 knockdown was evaluated by real-time PCR and western blotting.

Statistical analysis

All experiments were repeated five times. Data are expressed as the mean ± SD. Differences between group means were evaluated with the Mann-Whitney U test using SPSS v.15.0 software (SPSS Inc., Chicago, IL, USA). A P value < 0.05 was considered statistically significant.

III. RESULTS

TSA decreases viability and induces apoptosis in HESCs

Stromal cell monolayer cultures derived from eutopic and ectopic endometrium were established. The purity of HESCs was > 95%, as evidenced by vimentin and CD10 immunoreactivity and the absence of cytokeratin expression (Fig. 1).

HESCs viability was reduced in a dose-dependent manner by treatment with TSA, as determined by the MTT assay. Cells derived from eutopic endometrium were more sensitive to TSA treatment than those from ectopic endometrium (Fig. 2). The induction of apoptosis was determined by annexin V/PI double staining followed by flow cytometry. The percentage of early and late apoptotic HESCs was increased upon treatment with TSA, with a greater effect observed in eutopic than in ectopic HESCs (Fig. 3).

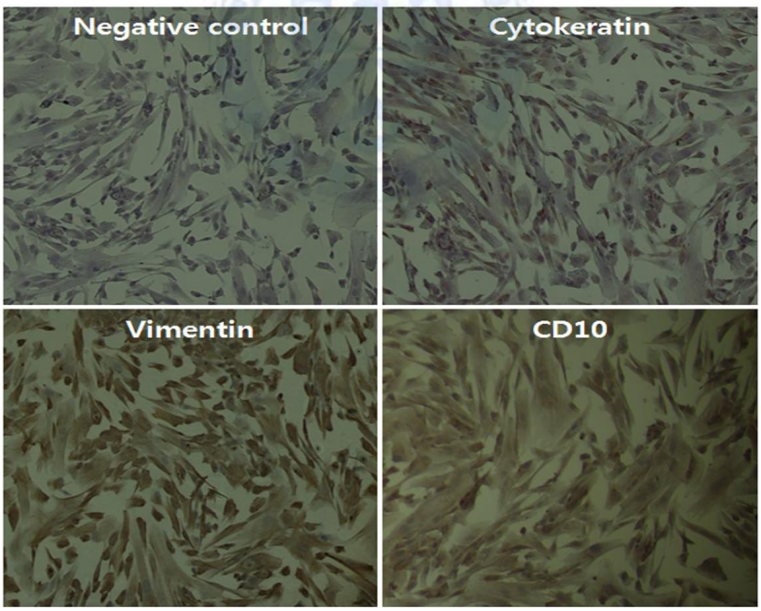

Figure 1. Immunocytochemical analysis of cytokeratin, vimentin, and cluster of differentiation (CD)10 expression in cultured ectopic HESCs. Cells were positive for vimentin and CD10 and negative for cytokeratin immunoreactivity.

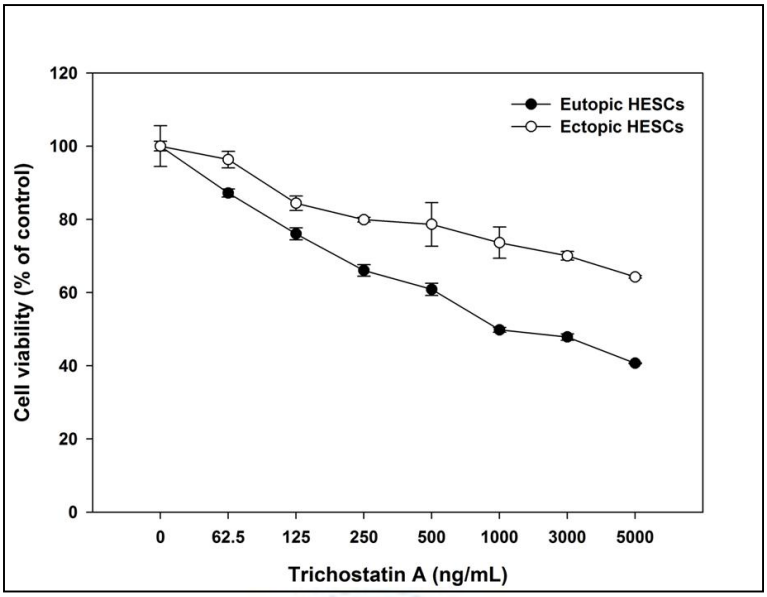

Figure 2. Viability of HESCs upon treatment with TSA. Cells were treated with indicated concentrations of TSA for 48 h and viability was assessed with the MTT assay. Data are expressed as mean ± SD of five independent experiments. MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; HESC, human endometrial stromal cells; TSA, trichostatin A.

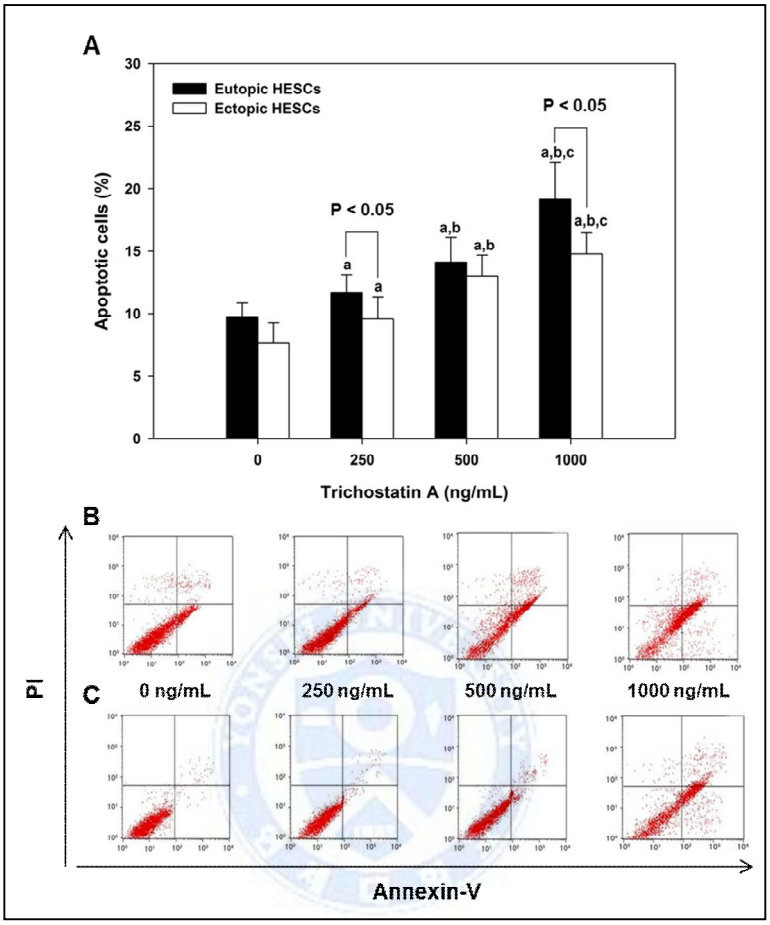

Figure 3. Effect of TSA treatment on HESC apoptosis (A). Apoptotic HESCs were identified by flow cytometry (B, eutopic HESCs; C, ectopic HESCs). Data are expressed as mean ± SD of five independent experiments. TSA, trichostatin A; HESC, human endometrial stromal cells. $^{a}P < 0.05$ versus 0 ng/mL, $^{b}P < 0.05$ versus 250 ng/mL, $^{c}P < 0.05$ versus 500 ng/mL.

NAG-1 expression is induced by TSA treatment

NAG-1 mRNA and protein expression was induced in a dose-dependent manner by TSA treatment at concentrations ranging from 500 to 5000 ng/ml for 48 h (Fig. 4). To determine whether NAG-1 expression is linked to HESC viability, NAG-1 expression was knocked down by transfecting cells with NAG-1 siRNA, followed by treatment with TSA. Suppressing NAG-1 expression abrogated the increase in cell viability induced by TSA (Fig. 5).

TSA does not act synergistically with a DNA demethylating agent

Treatment with various concentrations of 5-aza-dC reduced HESC viability in a dose-dependent manner, as determined by the MTT assay. Cells derived from eutopic endometrium were more sensitive to 5-aza-dC treatment than those from ectopic endometrium (Fig. 6). To assess whether combined treatment with 5-aza-dC can potentiate the apoptosis-inducing effects of TSA, HESCs were treated with vehicle (DMSO), 125 ng/ml TSA/12.5 μM 5-aza-dC alone or in combination, or 250 ng/ml TSA/25.0 μM 5-aza-dC alone or in combination. Cell viability was similar irrespective of whether cells were treated with both drugs concurrently or either drug alone (Fig. 7).

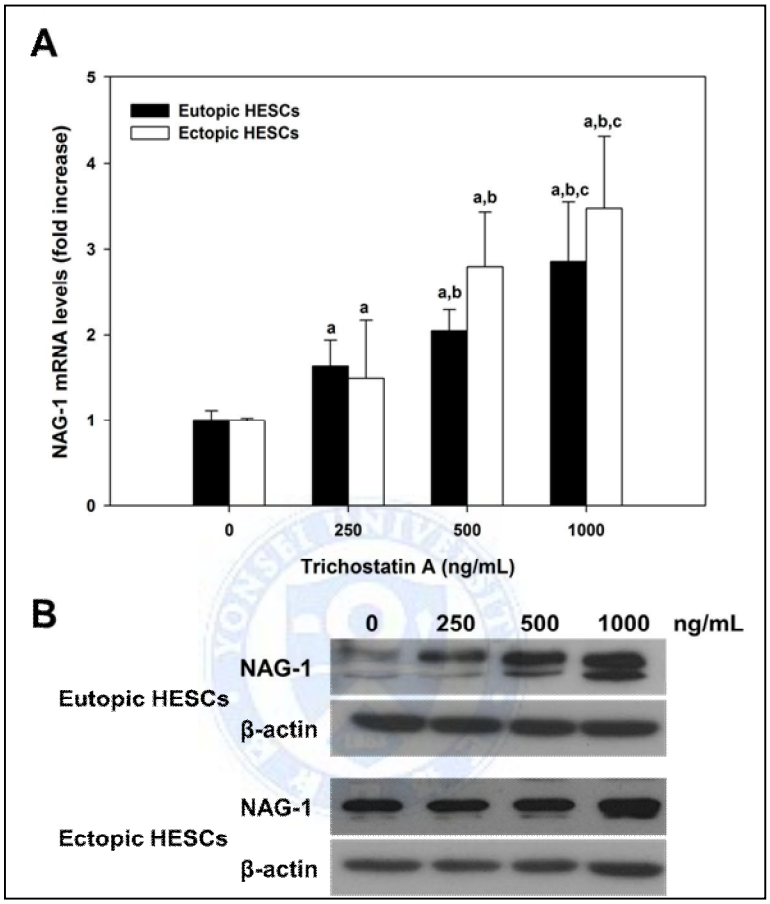

Figure 4. Effect of TSA on NAG-1 mRNA levels (A) and protein expression (B) in HESCs. TSA induced NAG-1 expression in a dose-dependent manner, as determined by RT-PCR and western blotting. β-actin served as a loading control for western blotting and was used to normalize mRNA expression level. Data are expressed as mean ± SD of five independent experiments. TSA, trichostatin A; HESC, human endometrial stromal cells. $^{a}P < 0.05$ versus 0 ng/mL, $^{b}P < 0.05$ versus 250 ng/mL, $^{c}P < 0.05$ versus 500 ng/mL.

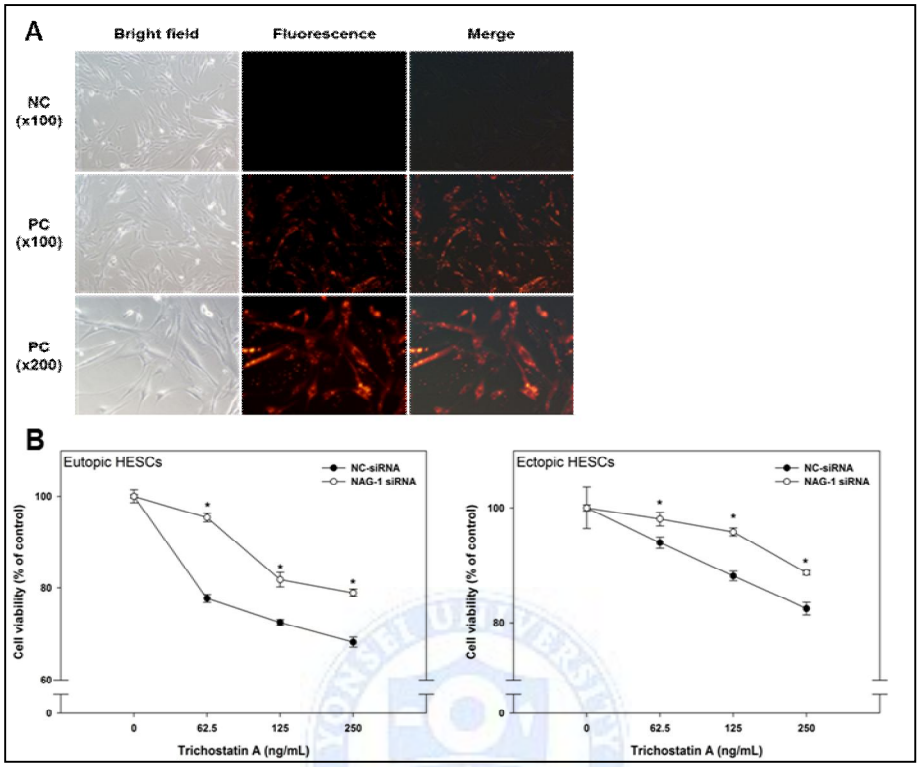

Figure 5. NAG-1 knockdown abrogates that TSA-induced increase in HESC apoptosis. HESCs were transfected with control or NAG-1 siRNA for 24 h, then treated with indicated concentrations of TSA or vehicle (DMSO) for 24 h. Cell viability was determined with the MTT assay. Data are expressed as mean ± SD of five independent experiments. MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; TSA, trichostatin A; HESC, human endometrial stromal cells. **P* < 0.01 versus NC-siRNA.

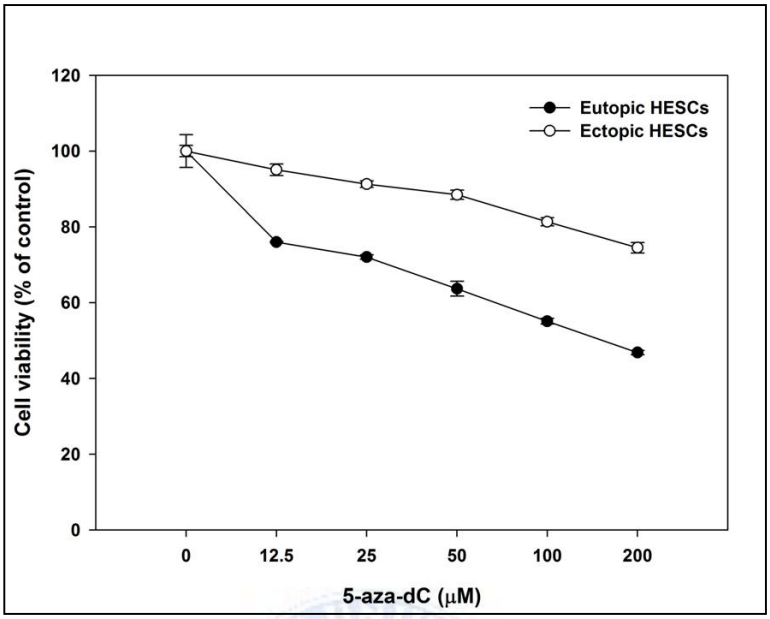

Figure 6. Viability of HESCs upon treatment with 5-aza-dC. Cells were treated with indicated concentrations of 5-aza-dC for 48 h and viability was determined with the MTT assay. Data are expressed as mean ± SD of five independent experiments. MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; HESC, human endometrial stromal cells; 5-aza-dC, 5-aza-2'-deoxycytidine.

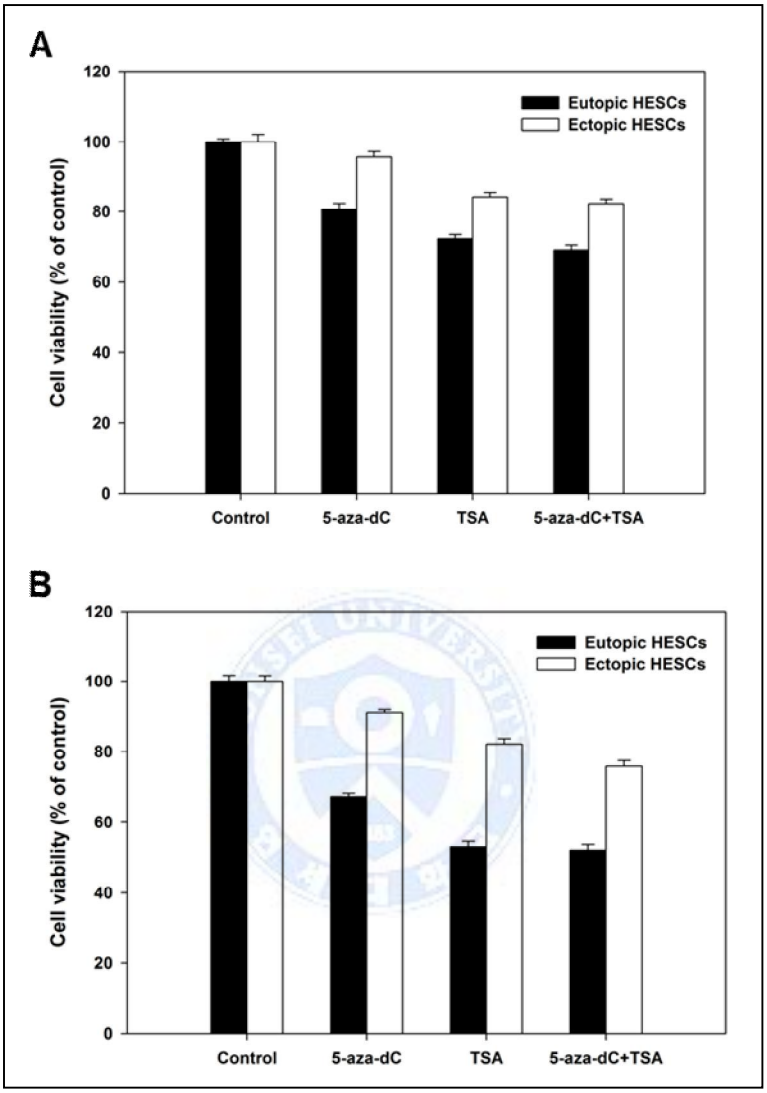

Figure 7. Effect of combined TSA/5-aza-dC treatment on HESC viability. HESCs were treated with vehicle (DMSO), 125 ng/ml TSA/12.5 μM 5-aza-dC alone or in combination (A) or 250 ng/ml TSA/25.0 μM 5-aza-dC alone or in combination (B). Cell viability was determined with the MTT assay. Data are expressed as mean ± SD of five independent experiments. MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; HESC, human endometrial stromal cells; TSA, trichostatin A; 5-aza-dC, 5-aza-2'-deoxycytidine.

IV. DISCUSSION

The results of this study demonstrate that TSA increases NAG-1 expression and induces apoptosis in HESCs. The knockdown of NAG-1 expression abolished TSA-induced apoptosis in HESCs, implying that this effect is mediated by NAG-1. However, TSA-induced apoptosis was unaffected by 5-aza-dC, suggesting that the two agents do not act synergistically.

Recent evidence indicates that endometriosis is an epigenetic disease. The expression levels of estrogen receptor (ER) α and β, progesterone receptor, and steroidogenic factor (SF)-1 is altered in endometriosis lesions.[14,15] Hypomethylation of the ERβ and SF-1 promoters results in pathological overexpression of the two proteins in ectopic HESCs, leading to progesterone resistance and disruption of estradiol inactivation.[15] In addition, the expression of homeobox (HOX)A10—a gene that is important for endometrial receptivity—is downregulated in the endometrium of women with endometriosis, which is likely due to aberrant methylation; additionally, treatment with a DNA-demethylating agent has been shown to increase HOXA10 expression in HESCs.[16] Thus, epigenetic aberrations may underlie the pathogenesis of endometriosis, which may accordingly be treated with DNA demethylating agents or HDACIs. In particular, HDACIs have been shown to induce growth arrest, differentiation, and apoptosis in HESCs and animal models of endometriosis.[4-6] Consistent with these findings, our study also found that TSA induced apoptosis in cultured HESCs in a dose-dependent manner.

NAG-1 overexpression in various types of cancer cell causes growth arrest and stimulates apoptosis, suggesting that NAG-1 has anti-tumorigenic and pro-apoptotic activities.[7,13,17] Our previous study showed cyclic expression of NAG-1 in endometrial glandular and stromal cells throughout the menstrual cycle, which was diminished in endometriosis patients. Induction of NAG-1 expression in HESCs also leads to apoptosis.[9] These observations suggest that downregulation of NAG-1 expression is associated with the establishment of endometriosis and that reversing this trend is a promising therapeutic approach for

endometriosis treatment. Based on our results, we presume that NAG-1 is an HDACI target that plays a critical role in HDACI-mediated apoptosis in HESCs. Only one other study has explored the link between TSA-induced apoptosis and NAG-1 expression.[13]

HDACIs have been reported to act synergistically with DNA-demethylating agents to potentiate the activation of silenced genes in various cancer cell lines.[18-20] In addition, HDACI-induced apoptosis was enhanced in the presence of a DNA-demethylating agent.[21] Although the underlying mechanism is unclear, this implies a functional link between DNA methylation and histone deacetylation in gene silencing.[21,22]

We observed that the apoptosis-inducing effects of TSA were greater in eutopic as compared to ectopic HESCs, implying that the latter are more resistant to TSA for reasons that are unclear. Combining drugs can reduce the pharmacological doses that are used in patients so that high efficacy can be achieved while minimizing toxicity; however, we did not observe synergy in the apoptosis-inducing effects of TSA and 5-aza-dC in ectopic HESCs. A previous study found that TSA combined with valproic acid did not have synergistic effects on cell cycle arrest in immortalized HESCs.[5] There is evidence to suggest that non-histone proteins, including transcription factors, are HDACI targets; therefore, the biological effects of HDACIs may be more varied than has been previously supposed.[23]

We have identified a novel mechanism for apoptosis induction by TSA in HESCs. Our results show a direct correlation between TSA-induced apoptosis and NAG-1 expression, which is known to inhibit tumorigenesis and induce apoptosis. Specifically, NAG-1 plays an important role in maintaining homeostasis in the endometrium; hence, aberrant expression of NAG-1 may contribute to the development of endometriosis.

This study had some limitations. We investigated the effects of only TSA, although several classes of HDACI have been described[23] that may have effects on HESCs that are different from those of TSA. Furthermore, we carried out only in vitro experiments; additional studies with animal models are required to confirm

the therapeutic potential of TSA in the treatment of endometriosis

V. CONCLUSION

TSA induced apoptosis in HESCs via induction of NAG-1 expression. These results suggest that TSA—and possibly other HDACIs or agents that induce NAG-1 expression—can be effective for treating endometriosis.

REFERENCES

1. Cramer DW, Missmer SA. The epidemiology of endometriosis. Ann N Y Acad Sci 2002;955:11-22.
2. Pritts EA, Taylor RN. An evidence-based evaluation of endometriosis associated infertility. Endocrinol Metab Clin North Am 2003;32:653-67.
3. Guo SW. Epigenetics of endometriosis. Mol Hum Reprod 2009;15:587-607.
4. Wu Y, Starzinski-Powitz A, Guo SW. Trichostatin A, a histone deacetylase inhibitor, attenuates invasiveness and reactivates E-cadherin expression in immortalized endometriotic cells. Reprod Sci 2007;14:374-82.
5. Wu Y, Guo SW. Histone deacetylase inhibitors trichostatin A and valproic acid induce cell cycle arrest and p21 expression in immortalized human endometrial stromal cells. Eur J Obstet Gynecol Reprod Biol 2008;137:198-203.
6. Lu Y, Nie J, Liu X, Zheng Y, Guo SW. Trichostatin A, a histone deacetylase inhibitor, reduces lesion growth and hyperalgesia in experimentally induced endometriosis in mice. Hum Reprod 2010;25:1014-25.
7. Baek SJ, Kim JS, Jackson FR, Eling TE, McEntee MF, Lee SH. Epicatechin gallate-induced expression of NAG-1 is associated with growth inhibition and apoptosis in colon cancer cells. Carcinogenesis 2004;25:2425-32.
8. Li PX, Wong J, Ayed A, Ngo D, Brade AM, Arrowsmith C, et al. Placental transforming growth factor-b is a downstream mediator of the growth arrest and apoptotic response of tumor cells to DNA damage and p53 overexpression. J Biol Chem 2000;275:20127-35.
9. Seo SK, Nam A, Jeon YE, Cho S, Choi YS, Lee BS. Expression and possible role of non-steroidal anti-inflammatory drug-activated gene-1 (NAG-1) in the human endometrium and endometriosis. Hum Reprod

2010;25:3043-9.

10. Baek SJ, Kim KS, Nixon JB, Wilson LG, Elling TE. Cyclooxygenase inhibitors regulate the expression of a TGF-b superfamily member that has proapoptotic and antitumorigenic activities. Mol Pharmacol 2001;83:493-501.
11. Yamaguchi K, Lee SH, Eling TE, Baek SJ. A novel peroxisome proliferator-activated receptor gamma ligand, MCC-555, induces apoptosis via posttranscriptional regulation of NAG-1 in colorectal cancer cells. Mol Cancer Ther 2006;5:1352-61.
12. Martinez JM, Sali T, Okazaki R, Anna C, Hollingshead M, Hose C, et al. Drug-induced expression of nonsteroidal anti-inflammatory drug-activated gene/macrophage inhibitory cytokine-1/prostate-derived factor, a putative tumor suppressor, inhibits tumor growth. J Pharmacol Exp Ther 2006;318:899-906.
13. Yoshioka H, Kamitani H, Watanabe T, Eling TE. Nonsteroidal anti-inflammatory drug-activated gene (NAG-1/GDF15) expression is increased by the histone deacetylase inhibitor trichostatin A. J Biol Chem 2008;283:33129-37.
14. Wu Y, Strawn E, Basir Z, Halverson G, Guo SW. Promoter hypermethylation of progesterone receptor isoform B (PR-B) in endometriosis. Epigenetics 2006;1:106-11.
15. Bulun SE. Endometriosis. N Engl J Med 2009;360:268-79.
16. Lu H, Yang X, Zhang Y, Lu R, Wang X. Epigenetic disorder may cause downregulation of HOXA10 in the eutopic endometrium of fertile women with endometriosis. Reprod Sci. 2013;20:78-84.
17. Kim JS, Baek SJ, Sali T, Eling TE. The conventional nonsteroidal anti-inflammatory drug sulindac sulfide arrests ovarian cancer cell growth via the expression of NAG-1/MIC-1/GDF-15. Mol Cancer Ther 2005;4:487-93.

18. Baylin S, Bestor TH. Altered methylation patterns in cancer cell genomes: cause or consequence? Cancer Cell 2002;1:299-305.
19. Baylin SB, Esteller M, Rountree MR, Bachman KE, Schuebel K, Herman JG. Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. Hum Mol Genet 2001;10:687-92.
20. Cameron EE, Bachman KE, Myöhänen S, Herman JG, Baylin SB. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nat Genet 1999;21:103-7.
21. Zhu WG, Lakshmanan RR, Beal MD, Otterson GA. DNA methyltransferase inhibition enhances apoptosis induced by histone deacetylase inhibitors. Cancer Res 2001;61:1327-33.
22. Vendetti FP, Rudin CM. Epigenetic therapy in non-small-cell lung cancer: targeting DNA methyltransferases and histone deacetylases. Expert Opin Biol Ther 2013;13:1273-85.
23. Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene 2007;26:5541-52.

ABSTRACT (IN KOREAN)

자궁내막증 환자의 자궁내막세포에서 trichostatin A에 의한 NAG-1의 발현과 세포자멸사의 변화

<지도교수 이병석>

연세대학교 대학원 의학과

서 석 교

목적: 본 연구에서는 자궁내막증 환자의 자궁내막세포에서 trichostatin A (TSA)에 의한 non-steroidal anti-inflammatory drug-activated gene-1 (NAG-1)의 발현과 세포자멸사의 변화를 알아보고자 하였으며, 5-aza-2'-deoxycytidine (5-aza-dC)이 TSA의 효과를 높일 수 있는지 알아보고자 하였다.

연구방법: 원소성 자궁내막조직은 근종으로 자궁절제술을 시행 받은 10명의 환자로부터 획득하였고 이소성 자궁내막조직은 자궁내막종으로 낭종절제술을 시행 받은 15명의 환자로부터 획득하였다. 획득한 자궁내막조직으로부터 간질세포를 분리한 후 세포배양을 시행하여 다양한 농도의 TSA를 처리하였다. NAG-1 mRNA의 농도는 실시간중합효소연쇄반응을 이용하여 정량화하였고 NAG-1 단백질의 발현은 웨스턴블롯을 이용하여 측정하였다. 세포자멸사는 흐름세포 측정법을 사용하였다.

결과: 자궁내막증 환자의 자궁내막간질세포에서 TSA의 농도에 비례하여 NAG-1의 발현과 세포자멸사가 증가하였다. TSA에 의한 세포자멸사는 NAG-1 siRNA를 이용하여 NAG-1 유전자의 발현을 억제하였을 때 감소하였다. 5-aza-dC는 TSA의 효과를 높이지 않았다.

결론: TSA가 자궁내막증 환자의 자궁내막세포에서 NAG-1 발현을 유도하여 세포자멸사를 유발한다. 이러한 결과는 TSA가 자궁내막증 치료에 유효할 것으로 생각되며, NAG-1은 자궁내막증 치료의 새로운 표적이 될 수 있을 것으로 생각된다.

핵심되는 말: 자궁내막증, 후생유전학, trichostatin A, NAG-1