High mobility group box-1 promotes proliferation and invasion in endometriotic stromal cells through Toll like receptor-4/Nuclear factor-kappa B

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Directed by Professor Seok Kyo Seo

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<TABLE OF CONTENTS>

LIST OF FIGURES

ABSTRACT

High mobility group box-1 promotes proliferation and invasion in endometriotic stromal cells through toll-like receptor-4/nuclear factor-kappa B

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The objective of this study was to evaluate whether high mobility group box (HMGB)-1 induces cell proliferation, invasion, and mediates inflammation in ectopic human endometrial stromal cells via toll-like receptor (TLR) 4 and the nuclear factor (NF)-κB pathway. An experimental study was performed in a tertiary university hospital and laboratory, in which ten women with ovarian endometriomas participated. Ectopic endometrial tissue from the endometriomas was collected. Human endometrial stromal cells (HESCs) were treated with recombinant HMGB-1 in a dose-dependent manner. Using real-time reverse transcription polymerase chain reaction (qRT-PCR) and western blotting, cell proliferation and invasion, TLR4 mRNA and proteins, receptors for advanced glycation end products (RAGE), and vessel endothelial growth factor (VEGF) were examined. The expression of mRNA for the adhesion molecules intracellular adhesion molecule-1 (ICAM-1) and E-cadherin were measured. Inflammatory cytokines were measured from the supernatants of HESCs in accordance with rHMGB-1 treatment. During rHMGB-1 treatment, expression of the TLR4 and

RAGE genes and proteins increased in HESCs. VEGF synthesis also increased under these circumstances. Gene expression of ICAM-1 was upregulated, whereas that of E-cadherin was downregulated with rHMGB-1 treatment. Inflammatory cytokine secretion increased significantly during rHMGB-1 treatment. After blocking TLR4 by siTLR4 transfection during rHMGB treatment, cell proliferation and invasion showed a marked decrease. HMGB-1 activates the NF-κB pathway via TLR4 to increase cell proliferation, invasion, and the production of various inflammatory markers.

Key words: Endometriosis, HMGB-1, DAMP, NF-κB, TLR4

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I. INTRODUCTION

Endometriosis is a chronic and multifactorial disease affecting reproductive function, and typically develops in women aged 30-40 years. As the research of endometriosis pathogenesis remains at the level of hypotheses, management is limited to conservative treatments such as pain control and fertility treatments. After reports of endometriosis patients presenting with increased levels of inflammatory cytokines in the peritoneal cavity surfaced in the early 1990s[1], the pathophysiology of the immune system has been suggested as a main factor in the development and progression of endometriosis.

Oxidative stress and damage-associated molecular patterns (DAMPs) are stimuli which activate the innate immune system in the human body, leading to a noninfectious inflammatory response. High mobility group box-1 (HMGB-1) is a representative DAMP, referred to as a prototype[2]. HMGB-1 exists as an intranuclear DNA binding protein in mammalian cells, stabilizing DNA and controlling transcriptional activity[3]. When released extracellularly via cell death, HMGB-1 acts as an endogenous ligand,

activating inflammatory pathways. Previously, we reported an increased release of HMGB-1 in human endometrial stromal cells (HESCs) by oxidative stress-induced cell death. Treatment of HESCs with rHMGB-1 revealed that this has a correlation with expression of its receptor, toll-like receptor 4 (TLR4). According to the TLR4 antagonist and nuclear factor-kappa B (NF-κB) inhibitor treatment, changes in HMGB-1 and TLR4 were consistently correlated. Thus, innate immune response via HMGB-1 and TLR4, as NF-κB induced alteration in naïve and eutopic endometrium, NF-κB was therefore suggested to be a possible contributor to endometriosis development. We concluded that HMGB-1 may have a role in the early development of endometriosis.

In the current study, we aimed to elucidate whether HMGB-1 induces cell proliferation, invasion, and mediates inflammation in ectopic human endometrial stromal cells via the TLR4 and NF-κB pathway, hence contributing to the development and progression of endometriosis.

II. MATERIALS AND METHODS

Participants

From October 2014 to March 2015, ectopic endometrial specimens were obtained from 10 patients with surgically diagnosed endometriosis. The ectopic endometrium was retrieved from ovarian endometriomas. All 10 patients were of reproductive age, and none of the participants received any hormonal treatment for at least 3 months preceding the surgery. The study was approved by the institutional review board of Severance Hospital, Yonsei University College of Medicine (4-2014-0560). The 10 participants who provided endometrial tissue for the cell culture provided written informed consent.

Sample collection

After the endometriotic cysts were enucleated, 1.0 × 1.0 × 1.0 cm^3 of tissue was

sampled from the cyst wall. The specimens were transferred into a phosphate-buffered saline solution(PBS) directly after acquisition and were immediately sent to the laboratory for analysis.

Cell culture

Isolated ectopic human endometrial stromal cells (HESCs) were cultured as described previously[4]. After washing and sectioning in PBS, they were treated with 0.25% trypsin/EDTA (Gibco, Invitrogen) for 30 min at 37 °C. During incubation, cells were tapped every 5 min to facilitate dissociation. Supernatant was removed via centrifugation at 2000 rpm for 5 min, and ectopic HESCs were resuspended in Dulbecco's modified Eagle's medium/F12 (DMEM/F12, HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Invitrogen). The stromal-enriched fraction was cultured in 75 mL culture flasks at 37 °C, in a humidified atmosphere of 95% air and 5% CO_2. HESCs used for the experiments were cells with a passage of 2 to 6.

Cell treatment & study design

To present ectopic HESCs with oxidative stress, endometrial cells were incubated with 0, 0.01, 0.1, or 1 nM H_2O_2 for 48 hours. After inducing cell necrosis via oxidative stress, the proliferation of ectopic HESCs and passive release of HMGB-1 were examined via an MTT assay. To observe the influence of DAMP on ectopic HESCs, endometrial cells were incubated with 0, 5, and 10 μM recombinant HMGB-1 (rHMGB-1, Sino, Beijing, China) for 48 hours. The expression of mRNA and TLR4 protein, receptor for advanced glycation end products (RAGE), vessel endothelial growth factor (VEGF), mRNA for adhesion molecules intracellular adhesion molecule-1 (ICAM-1) and E-cadherin, and inflammatory cytokines were measured via an invasion assay. This was done in accordance with rHMGB-1 treatment. Further, TLR4 was inhibited by small interfering RNA (siRNA) transfection. rHMGB-1 treatment was

performed thereafter, and cell migration was counted to examine changes in cell invasion.

Cell proliferation assays (CCK-8)

Ectopic HESCs were seeded in 6-well tissue culture plates at a density of 1×10^5 cells per well. The culture medium was changed to DMEM/F12 with 2% FBS after 24 h of incubation. After changing the media, endometrial cells were treated with 0, 0.01, 0.1, or 1 nM H_2O_2 for 48 h. Thereafter, 100 μL of CCK-8 (Cell Counting Kit-8; Dojindo, Japan) was added to each well, and plates were incubated at 37 °C for 1 h. Supernatants were transferred to 96-well plates after incubation, and the OD was measured at 450 nm using a VersaMax microplate reader (Molecular Devices) to measure cell proliferation rates.

Measurement of passive HMGB-1 release after cell death

For measurement of passive HMGB-1 release, 3×10^5 endometrial cells were plated in 6-well plates. After 24 h of incubation at 37 °C, the medium was changed to serum-free DMEM/F12. Endometrial cells were then incubated with 0, 50, or 100 μM H_2O_2 for 1 h. MTT solution (100 μL, Sigma) was added to each well, and culture plates were incubated at 37 °C for 4 h in a 5% CO_2 incubator. After removing the medium, 500 μL dimethyl sulfoxide (DMSO; Sigma) was added to each well, and plates were then incubated for 10 min on a shaker. Finally, the optical density (OD) was measured at 562 nm with a VersaMax microplate reader (Molecular devices, Sunnyvale, CA, USA). The supernatants were harvested for subsequent western blotting to examine HMGB-1 expression.

Invasion assay

Transwell matrigel chambers (Corning Inc., Lowell, MA, USA) were used to perform the invasion assay. Ectopic HESCs were seeded onto the upper matrigel

chamber with serum-free DMEM. The lower chamber contained DMEM with 20% FBS and rHMGB-1 at concentrations of 0, 5, and 10 ng/mL. After 48 hours, the cells that invaded the lower surface were collected. The invasive cells were stained and then counted under a microscope.

Real-time Polymerase Chain Reaction (RT-PCR)

Cell-free total RNA was extracted from cell lysates using an RNeasy mini kit (Qiagen, Hilden, Germany). cDNA was synthesized from 1 μg total RNA using oligo-dT primers (Invitrogen). RT-PCR for TLR4 was carried out using an ABI StepOnePlus (Applied Biosystems, Foster City, CA, USA) and SYBR green RT-PCR master mix (Toyobo, Osaka, Japan). The primers used for TLR4 were as follows: 5′-CAGAGTTTCCTGCAATGGATCA-3′ (sense) and 5′-GCTTATCTGAAGGTGTTGCACAT-3′ (antisense), and the primers for VEGF were: 5'- GGA GGAGGGCAGAATCATCAC-3' (sense) and 5'-ATTGGATGGCAGTAGCTGCCT-3'(antisense). The primers for E-cadherin were: 5'-TCATGAGTGTCCCCCGGTAT-3' (sense) and 5'- TCTTGAAGCGATTGCCCCAT-3' (antisense). The primers for ICAM-1 were: 5'-TCT TCC TCG GCC TTC CCA TA-3' (sense), 5'-AGG TAC CAT GGC CCC AAA TG-3' (antisense). Primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was used as an endogenous reference gene, were as follows: 5′-TCGACAGTCAGCCGCATCTTCTTT-3′ (sense) and 5′-ACCAAATCCGTTGACTCCGACCTT-3′ (antisense). The PCR conditions were as follows: 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Data were normalized to the expression of GAPDH, and PCR products were separated on a 1.5% agarose gel containing ethidium bromide. This was done for quantification by densitometry using Image J software (National Institutes of Health, USA).

Western blotting

Cells were lysed using radioimmunoprecipitation assay buffer (RIPA buffer; Intron, iNtRON Biotechnology, Sungnam, Korea) containing protease inhibitor cocktail (Cell Signaling Technology, Beverly, MA, USA). This was mixed and divided into 40 μL aliquots. The lysates were collected and centrifuged at 13000 rpm at 4 °C for 30 min, and the supernatant protein levels were quantified using a BCA protein assay kit (Thermo Scientific, Hudson, NH, USA). Western blotting was then performed using the harvested supernatants. Thirty micrograms of each lysate were boiled in 5× buffer, and supernatants were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 8% gels. They were then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Eschborn, Germany). After blocking with BSA at room temperature for 1 h, the membranes were incubated with primary antibodies specific for TLR4 (polyclonal anti-rabbit, 0.3 μg/mL, GeneTex, Irvine, CA, USA), VEGF (polyclonal anti-rabbit, 1 μg/mL, Abcam, Cambridge, UK), E-cadherin (monoclonal anti-rabbit, Cell Signaling Technology, Beverly, MA, USA), and GAPDH (monoclonal anti-mouse, 0.1 μg/mL, Millipore) overnight at 4 °C. The membranes were then incubated with anti-mouse antibodies (IgG, 0.27 μg/mL, Jackson, West Grove, PA, USA) and anti-rabbit antibodies (IgG, 0.27 μg/mL, Jackson) conjugated with horseradish peroxidase (HRP) for 1 h at room temperature. Detection was facilitated by enhanced chemiluminescence (ECL) solution (Advansta, San Francisco, CA, USA), and the bands were quantified by densitometry using Image J software.

ELISA

After treating ectopic HESCs with 0, 5, or 10 ng/mL rHMGB-1 for 48 hours, the supernatant was harvested for ELISA. Supernatants were analyzed for IL-6 (BD bioscience, San Jose, CA, USA), TNF-α (GE healthcare, Buckinghamshire, UK), IL-1β (Abcam, Cambridge, UK), and IL-10 (Abcam, Cambridge, UK) using an ELISA kit,

according to the manufactures' protocol. The OD was measured at 450 nm with an ELISA Reader.

Statistical analysis

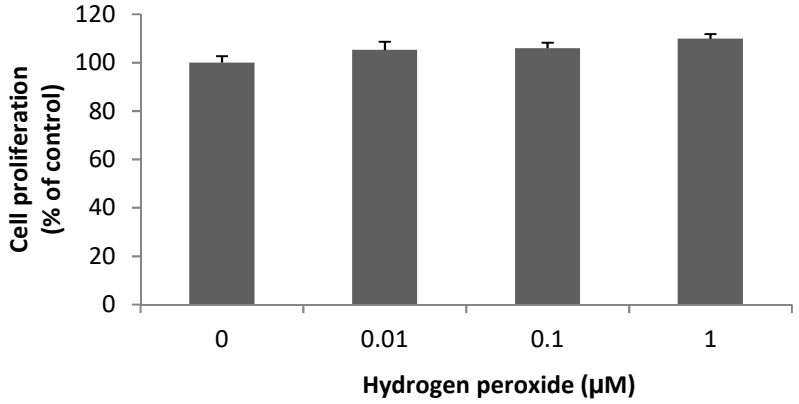
To determine the significance of the difference in the mRNA and protein levels, Kruskal-Wallis tests with Dunn's procedure for multiple comparisons were performed. Statistical analyses were performed using SPSS 23.0 (IBM, NY, USA). Differences with P values of less than 0.05 were considered statistically significant.

III. RESULTS

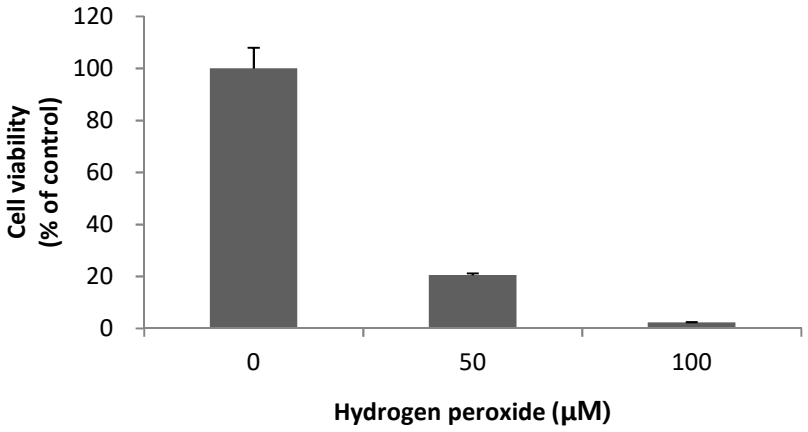
1. Cell proliferation and HMGB-1 release after H_2O_2 treatment

To apply oxidative stress to ectopic HESCs, the cells were treated with 0.01, 0.1, and 1 nM H_2O_2. According to the results, there was a gradual increase in cell proliferation (Figure 1-A). During the MTT assay, significant cell death was demonstrated by an increase in H_2O_2 concentration (Figure 1-B) and in the passive release of HMGB-1, in correlation with cell death (Figure 1-C).

(A)



(B)



(C)

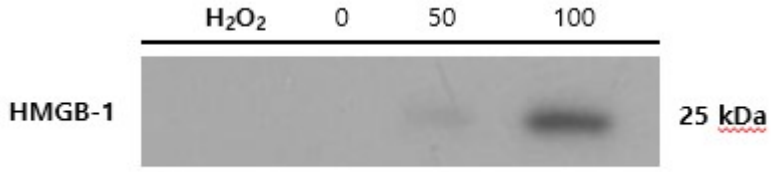
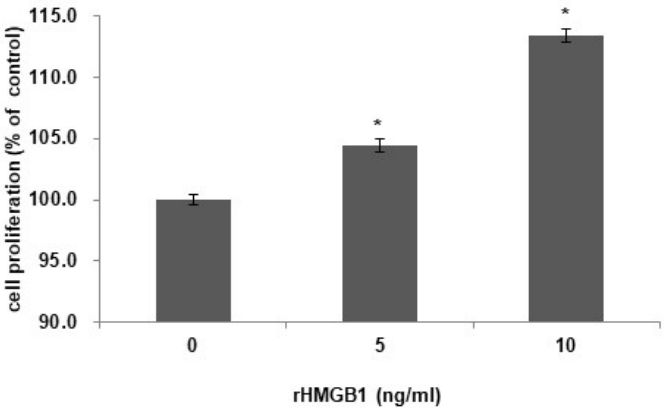


Figure 1. Oxidative stress caused cell proliferation (A), decreased cell viability (B), and increased HMGB-1 release (C) in ectopic HESCs.

2. Changes in ectopic HESCs after treating with rHMGB-1

When treatment with rHMGB-1 was used as a form of oxidative stress instead of H_2O_2, cell proliferation and cell invasion increased according to the treatment dose and time (Figure 2-A). To examine changes in cell invasion, treatment of ectopic HESCs with rHMGB-1 was done in a dose- and time-dependent manner. As the treatment time and rHMGB-1 dose increased, cell invasion significantly increased according to these changes (Figure 2-B). Moreover, in accordance with the increased rHMGB-1 dosage, TLR4 and RAGE showed marked increases of mRNA (data not shown) and protein expression (Figure 3).

(A)



(B)

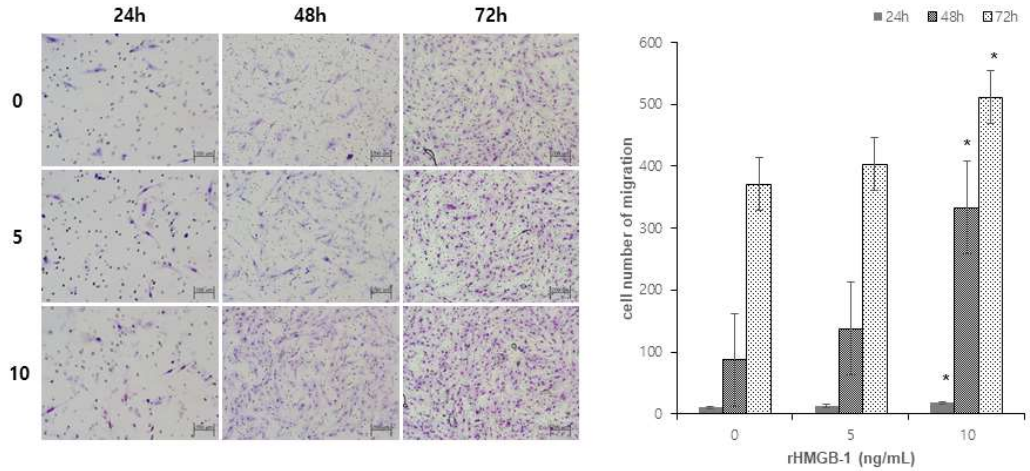


Figure 2. Cell proliferation (A) and the invasion assay (B) after inducing oxidative stress by treating ectopic HESCs with rHMGB-1. The migrating cell number significantly increased in each culture group (24 h, 48 h, and 72 h) when comparing rHMGB-1 doses of 0 ng/mL and 10 ng/mL.

*, $P < 0.05$ compared to rHMGB-1 0 ng/mL treatment.

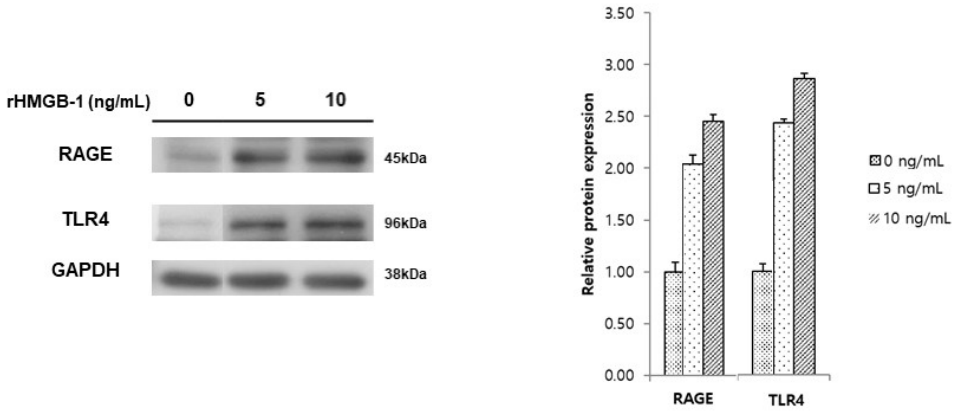


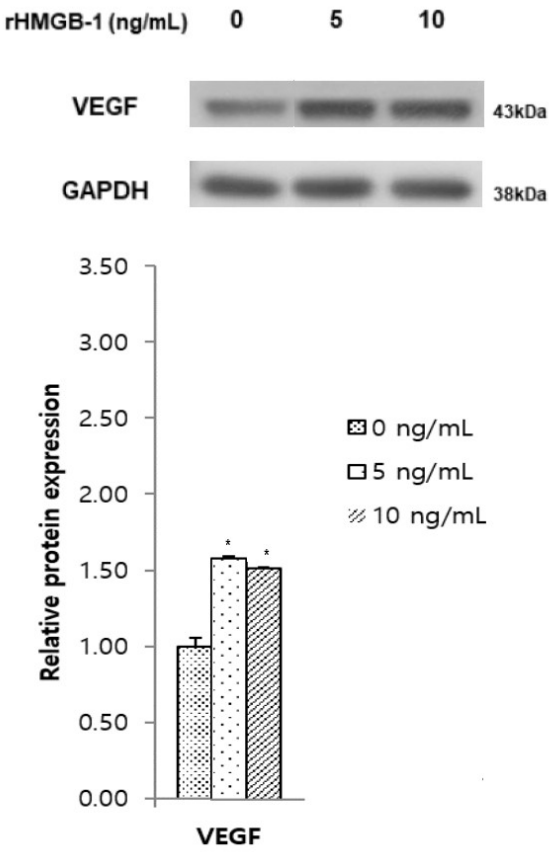
Figure 3. Increased expression of receptors for HMGB-1 according to the treatment

dosages (48 h). RAGE and TLR4 expression showed significant increases in an rHMGB-1 dose-dependent manner.

3. Changes of adhesion and angiogenic factors after rHMGB-1 treatment

After applying oxidative stress via rHMGB-1 treatment in a dose-dependent manner for 48 h, the angiogenic marker VEGF showed markedly increased mRNA and protein expression (Figure 4-A). Another angiogenic marker, ICAM-1, also showed a significant increase in mRNA expression when treated with 10 ng/mL rHMGB-1 compared to the naïve cells. Expression of adhesion marker E-cadherin was significantly inhibited by rHMGB-1 treatment (Figure 4-B).

(A)



(B)

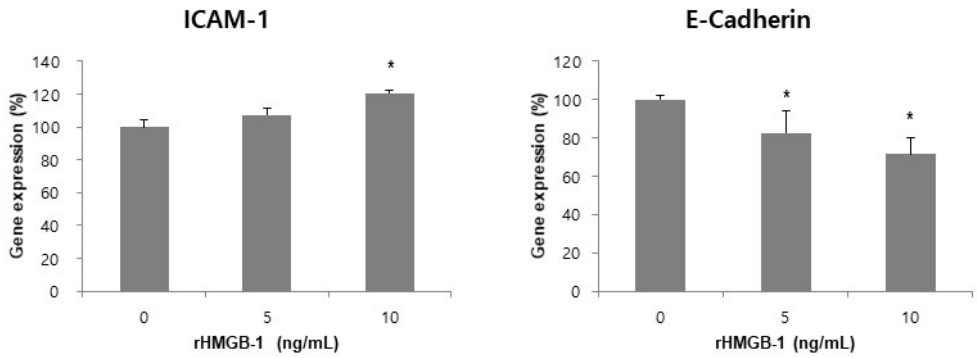


Figure 4. Protein expression of VEGF (A) and mRNA expression of ICAM-1 and E-cadherin (B) after rHMGB-1 treatment (48 h).

(A) VEGF expression was significantly increased by increased rHMGB-1 compared to the cell treated with 0 ng/mL.

(B) ICAM-1 mRNA expression increased only when treated with 10 ng/mL of rHMGB-1, whereas E-cadherin showed significant decreases when treated with rHMGB-1 in a dose-dependent manner.

*, $P < 0.05$ compared to treatment with 0 ng/mL rHMGB-1.

4. Inflammatory cytokine secretion in HESC supernatants according to rHMGB-1 treatment

After applying oxidative stress via dose-dependent rHMGB-1 treatment for 48 h, inflammatory cytokine levels were measured from the supernatants of the HESCs (Figure 5). IL-6 and IL-10 were serially increased according to increased rhMGB-1 doses. TNF-α and IL-1β were significantly secreted when administered with a higher dose of rHMGB-1.

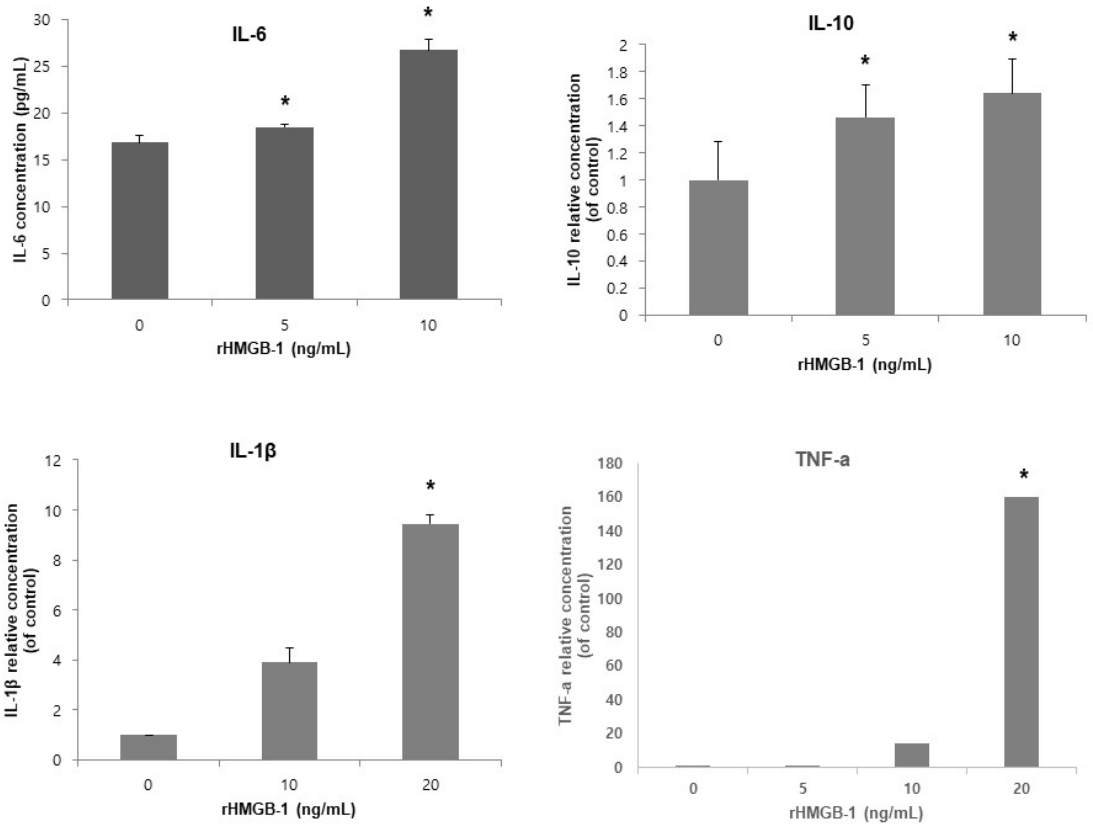


Figure 5. Inflammatory cytokine secretion in ectopic HESCs after rHMGB-1 treatment. *, $P < 0.05$ compared to treatment with 0 ng/mL rHMGB-1.

5. Cell invasion decreased after TLR4 inhibition by siRNA transfection

To examine the role of TLR4, TLR4 was knocked out by siTLR4 transfection. After siTLR4 transfection, treatment with rHMGB-1 was performed in the same manner as previously discussed with naïve HESCs. However, cell migration significantly decreased according to the increase in rHMGB-1 dosage (Figure 6).

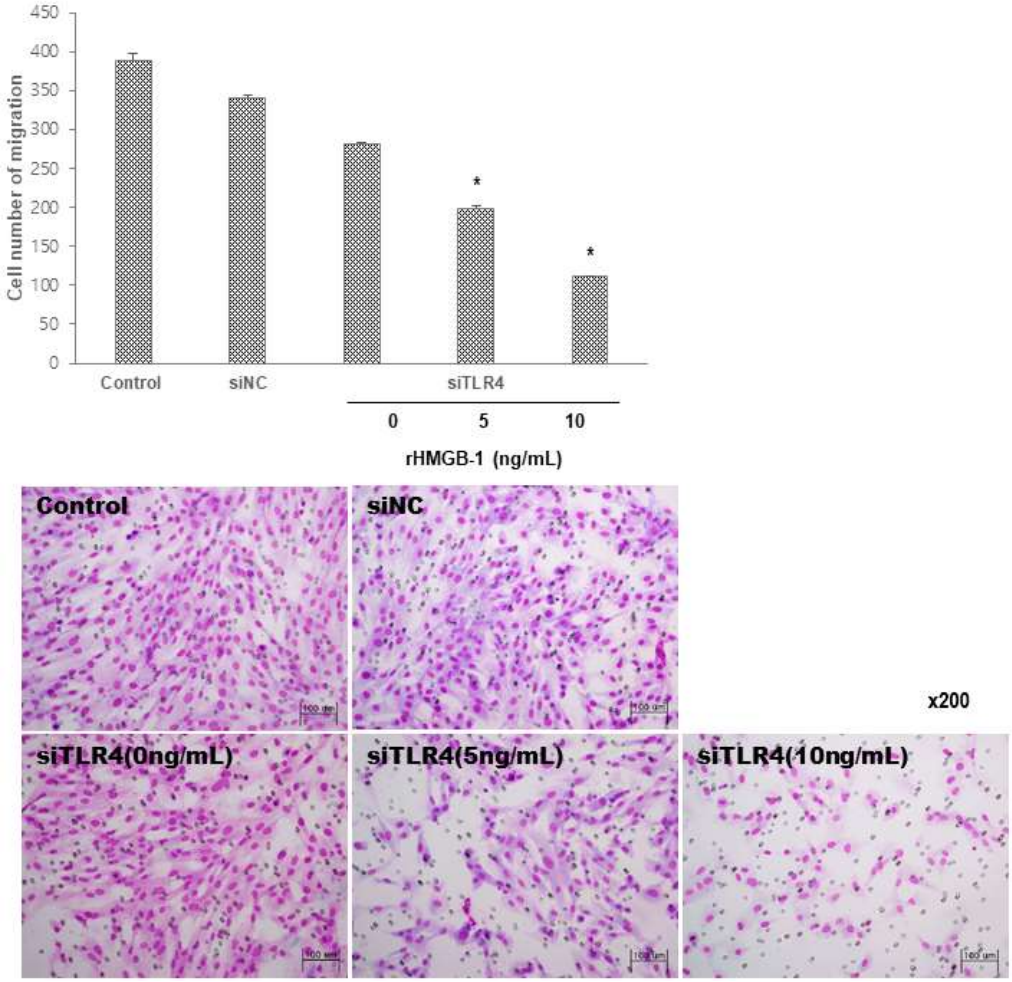


Figure 6. Cell invasion assay after treating siTLR4-transfected HESCs with rHMGB-1. Cell migration significantly decreased when treated with 5 ng/mL and 10 ng/mL of rHMGB-1 in these cells, compared to the 0 ng/mL treatment group.

*, $P < 0.05$ compared to treatment with 0 ng/mL rHMGB-1.

IV. DISCUSSION

In the current study, we developed an outline of the inflammatory process mediated

by the NF-κB pathway in endometriosis. This shows an increase in cell proliferation and invasion, adhesion molecules, angiogenesis, and in the secretion of cytokines via TLR4 when treated with rHMGB-1. Although we did not examine NF-κB itself, we have investigated cell migration and invasion, proliferation, angiogenic factor, adhesion molecules, inflammatory cytokines resulting from activation of the NF-κB pathway. By using ectopic HESCs, we sought to examine whether the danger signal may affect modified endometrium in addition to eutopic endometrium[5]. As an extension of our previous study[5], the passive release of HMGB-1 from dying ectopic HESCs by H_2O_2-induced hypoxic damage was shown. By using a DAMP such as rHMGB-1 as a stressor, cell proliferation and invasion also increased, suggesting active secretion of HMGB-1 by endogenous danger signals. Proportionately, the expression of RAGE and TLR4, the known receptors for HMGB-1, increased according to the DAMP stimuli in ectopic HESCs. By down-regulating TLR4 via siRNA transfection, cell proliferation and migration significantly decreased, suggesting that DAMP acts via TLR4 in ectopic HESCs.

As a possible method of activating the NF-κB pathway, innate immunity-related chronic inflammation has been suggested as a possible pathophysiology in endometriosis development[6]. NF-κB has been extensively studied in the field of endometriosis research[7,8] as well as in relation to other diseases[9]. NF-κB mediates the induction of pro-inflammatory genes, including cytokines, chemokines, and other inflammatory mediators. This occurs in various immune cells, such as macrophages, dendritic cells, and T and B lymphocytes. In endometriosis, an inflammatory pattern of shifting to Th2 from Th1 has been suggested[10]. Aberrantly regulated immune factors such as increased cytokines (TNF-α, IL-1β, IL-8, IL-6, IL-10)[11-13] and decreased natural killer cells in the peritoneal fluid[14] have been detected. Interest has turned to NF-κB pathway accordingly, and a few investigations have revealed increased expression of NF-κB subunits in the endometrium[7,15] and activation in macrophages[16].

As the NF-κB pathway has also been widely discussed for its role in carcinogenesis, autoimmune diseases, and inflammatory diseases, possible stimuli which may activate the NF-κB pathway in endometriosis have emerged. These stimuli include hypoxia, oxidative stress, DAMPs, microbium, iron burden, inflammasomes, and cytokines. It has been suggested that these may lead to NF-κB pathway activation, resulting in increased cell proliferation, decreased apoptosis, and inflammation modulation[6,8,17,18]. In our study, we sought to elucidate a pathophysiologic stream involved in endometriosis in terms of innate immunity. This involved DAMPs and the NF-κB pathway, which were previously known to be a scattered pattern. TLR4 down-regulation by siRNA transfection showed how cell migration and invasion changes when the receptor has been knocked down. We demonstrated eutopic endometrial stomal cells as naïve before the pathologic modification during endometriosis, and ectopic endometrial stromal cells post-modification by pathologic changes. Previous studies have demonstrated different proliferation, decidualization, and survival rates of stromal cells isolated from the eutopic endometrium, ovarian endometrioma, and deep infiltrating endometriosis. This suggests that different origins may reflect the cells' differing characteristics in endometriosis[19,20].

HMGB-1 is stored in the nucleus, and is a DNA binding protein which participates in DNA replication, recombination, transcription, and repair[2,21]. However, when HMGB-1 is released extracellularly, it functions as a signaling molecule in the inflammation pathway, immune system, and tissue regeneration. This includes cell differentiation and migration[3]. Extracellular HMGB-1 can be actively secreted by inflammatory cells stimulated by DAMPs, cytokines, or chemokines, or passively released by dead, dying, or injured cells. This can be done via several different mechanisms, as indicated[22]. Extracellular HMGB-1 acts as a DAMP molecule, and its activity depends on the redox state, receptors, and their interactive partners. HMGB-1 was suggested to be a key molecule in inducing the chronic inflammatory in rheumatic arthritis[23], retinopathy[24], sepsis[25], atherosclerosis[26,27], and liver diseases[22]. In atherosclerosis and liver disease in

particular, there are other features in common with endometriosis. These include increased stromal fibrosis, in addition to chronic inflammation. In non-alcoholic fatty liver disease (NAFLD), the suggested pathogenesis is the 'two-hit' hypothesis[28]. In this theory, when the liver is sensitized to injuries via steatosis, it is hit secondarily by cytokines, chemokines, and oxidative stress. This results in de novo lipogenesis and increased lipid deposition in the liver. HMGB-1-TLR4-MyD88 signaling has been thought to be a key mechanism in the early stages of NAFLD progression[29]. In NAFLD, HMGB-1 released by injured hepatocytes activates the NF-κB pathway via TLR4 and MyD88, leading to hepatic stellate cell activation and increased expression and synthesis of a-SMA and collagen, hence resulting in liver fibrosis[22].

Accordingly, our results show the possible passive release and active secretion of HMGB-1 in ectopic endometriotic cells, which may activate the NF-κB pathway via TLR4. Activation of NF-κB led to decreased expression of E-cadherin, increased ICAM-1 and VEGF expression, and concomitant proinflammatory and anti-inflammatory cytokine secretion. The observed increased VEGF[30] and ICAM-1[31] expression is in accordance with previous studies, as with the cytokine results. The attachment marker E-cadherin is important for maintaining cell to cell adhesion and cell polarity, and its decreased expression in endometriosis has been reported in various stages[32]. However, our study showed limitations in regards to performing only in vitro experiments. Furthermore, a suggested role of HMGB-1-TLR4-NF-κB during early endometriosis development needs to be evaluated and validated via in vivo study. Although siRNA transfection is a popular method used in endometrial cell experiments, an off-target signature on an unexpected target of the pathway may exist. As the objective for our study was to determine a pathway stimulated via TLR4, the results showed changes pre- and post-down-regulation. However, our study has strength that scarcity in terms of serial demonstration of a possible pathogenesis in endometriosis.

V. CONCLUSION

In conclusion, our study suggested that HMGB-1 may activate the NF-κB pathway via TLR4, resulting in inflammatory changes in ectopic HESCs. During the development of endometriosis, innate immunity may play an important role via this mechanism.

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ABSTRACT (IN KOREAN)

자궁내막증 기질세포에서 HMGB-1, TLR-4, NF-kB 경로를 통한 세포 증식 및 침윤 증가

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윤 보 현

본 연구는 인간 이소성 자궁내막기질세포에서 high mobility group box(HMGB)-1이 세포 증식 및 침윤 증가, 염증성 변화를 toll-like receptor(TLR)-nuclear factor(NF)-kB 경로를 통해 매개하는지 확인하고자 하였다. 3차 대학병원과 연구실에서 실험연구로 진행하였고, 10명의 난소 자궁내막종 환자가 참여하였다. 이소성 자궁내막세포를 자궁내막종 조직에서 채취하였고, 이를 이용한 원발성 세포 배양으로 세포를 얻었다. 인간 자궁내막기질세포(HESCs)는 recombinant HMGB-1으로 농도를 다르게 처리하였고, 이에 따른 세포 증식과 침윤, TLR4, receptor for advanced glycation end products (RAGE), vessel endothelial growth factor (VEGF)의 mRNA와 단백질 발현을 정량하였다. 또한 adhesion molecules-intracellular adhesion molecule-1 (ICAM-1)과 E-cadherin의 mRNA 발현과, 세포배양 부유액에서 염증성 사이토카인을 ELISA로 측정하였다. rHMGB-1의 처리에 따라, 이소성 HESCs에서 TLR4와 RAGE의 발현은 증가하였고, VEGF의

합성도 증가하였다.rHMGB-1의 처리 농도 증가에 따라 ICAM-1의 유전자 발현은 상승하였고, E-Cadherin의 발현은 감소하였다. 염증성 사이토카인의 분비 또한 rHMGB-1의 농도 증가에 따라 유의하게 증가하였다. TLR4를 siTLR4 transfection을 통해 차단하였을 때, HESCs 세포 증식과 침윤은 rHMGB-1을 처리함에 따라 유의한 감소를 보였다. 본 결과들을 종합할 때, HMGB-1은 TLR4를 통해 NF-kB 경로를 활성화해 자궁내막기질세포의 증식과 침윤 및 염증성 변화를 유발하여 자궁내막증의 발생에 기여하는 것으로 생각해볼 수 있다.

-핵심되는 말: 자궁내막증, HMGB-1, DAMP, NF-kB, TLR4