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석 사 학 위 논 문

Expression of Long Non-Coding RNAs
Induced by IL-1 β is Changed
by Inhibiting I κ B α Phosphorylation

계 명 대 학 교 대 학 원
의 학 과

강 현 주

지도교수 김상현 · 김지민

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이 논문을 석사학위 논문으로 제출함

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계 명 대 학 교 대 학 원

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Table of Contents

1. Introduction	1
2. Materials and Methods	4
3. Results	9
4. Discussion	18
5. Summary	23
References	24
Abstract	33
국문초록	35

List of Tables

Table 1. Primer Sequences for Validation of LncRNAs	8
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List of Figures

Figure 1. Hierarchical clustering heatmap and volcano plot analysis of differentially expressed lncRNAs	12
Figure 2. Comparison of lncRNA expression profiles between RA FLS and RA FLS treated with IL-1 β	13
Figure 3. GO functional analysis of GO terms for differentially expressed lncRNAs	14
Figure 4. Validation of upregulated lncRNAs using RT-qPCR	15
Figure 5. Effect of IMD-0560 on IL-1 β induced phosphorylation of I κ B α	16
Figure 6. LncRNAs upregulated by IL-1 β are downregulated by inhibiting I κ B α phosphorylation	17

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease with a slow onset of symptoms, mainly affecting the joints. Most commonly, the wrists and hands are affected, and usually, the same joints are used on both sides of the body (1). RA is an important health issue because it has a large financial and psychological burden on patients. As such, its early diagnosis, and timely treatment hold a great significance for preventing RA progression. Despite the development of therapies, many patients experience persistent inflammation and progressive disorders (2). However, the specific etiology of RA is unknown. The symptoms of RA range from slowly progressing to severely destructive diseases associated with inflammation (3). Although progress has been made in understanding the pathogenesis of RA, the exact mechanism of action still needs to be explained. Research increasingly shows that the inflammatory response plays a key role in RA. One of them, interleukin (IL) -1, is known to be an important pro-inflammatory cytokine and helps to understand the pathogenesis of RA (4). IL-1 is exerts effects on various cells and plays important roles in autoimmune disorders and inflammation (5). Pathophysiological changes occur during the disease phase. Autoimmune diseases such as rheumatoid arthritis, osteoarthritis, and inflammatory bowel disease are involved in the overproduction of IL-1 (6). IL-1 is also known to be under the control of the nuclear factor-kappa B (NF- κ B) transcription factor. In addition, the pro-inflammatory cytokines IL-6 and tumor necrosis factor (TNF) - α are involved in the NF- κ B pathway (7).

NF- κ B is already well known to be a major factor in the regulation

of inflammation and immunity in cancer progression. NF- κ B is known to be involved in canonical and non-canonical pathways, but the canonical pathway is usually involved (8). Pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α induce the NF- κ B pathway by activating inhibitors of the NF- κ B (I κ B) kinase (IKK). The contribution of the NF- κ B canonical pathway to acute inflammation and cell-survival mechanisms is well accepted (9). Activation of NF- κ B in the synovial membrane of RA patients is much higher than in healthy subjects or osteoarthritis patients. The expression of NF- κ B results in the expression of inflammatory molecules, including adhesion molecules, cytokines, and chemokines, which play a key role in the development of RA. NF- κ B is considered a target in RA treatment, being used for improving the incidence of joint destruction (8).

Long non-coding RNAs (lncRNAs) are a type of RNA molecule that is more than 200 nucleotides in length without producing protein. The role of non-coding RNA has recently been reported in several disease processes, including cancer (10). Initially, small regulatory RNAs have been extensively studied, such that their underlying molecular mechanisms are well known (11). Although the specific function of lncRNA is not largely known, recent studies have revealed the potential for involvement in a wide range of biological processes (12). However, the clear role of lncRNAs in the pathogenesis of autoimmune disease unclear. Despite this, many lncRNAs have been found to play many roles in the pathogenesis of immune-mediated inflammatory diseases (13). One study found that the reduction of long intergenic non-coding RNA-p21 in RA contributes to increased NF- κ B activity (14). Also, lncRNA-Cox2 has been reported to play an important role in the activation and inhibition of the expression of immune regulatory genes. Thus, lncRNA-Cox2 may affect the development

of autoimmune diseases (15).

In this study, I found out lncRNAs influenced by IL-1 β , a mediator in the pathogenesis of RA, using Next Generation Sequencing (NGS). Whether the regulation of NF- κ B activation, which is known to be induced by IL-1 β , resulted in changes in expression of these lncRNAs was investigated.

2. Materials and Methods

2.1. Patients and Sample:

This research was conducted with the approval from the Human Ethics Committee Dongsan Medical Center of Keimyung University Institutional Review Board (IRB) (DMSC 2016-06-010-012). Fibroblast-like synoviocytes (FLS) were obtained after joint surgery in RA patients. Samples were collected from patients with written consent. All patients met the American Society of Rheumatology/European Rheumatology (ACR/EULAR) criteria revised in 2010 (14).

2.2. Extraction and Culture of FLS:

To obtain FLS, tissue samples were first chopped using scissors and digested with 2 mg/ml type II collagenase (Invitrogen, Carlsbad, CA, USA) at 37 °C for 2 h (16). The cells were pelleted after centrifugation for 5 min at 2000 rpm. After leaving only the pellet and suctioning the supernatant, the cells were resuspended in high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, before culturing in a 100-mm dish in 5% CO₂ incubator at 37 °C for 24 h. Passages 3-5 cells were used for all experiments.

2.3. RA FLS Treated with IL-1 β :

RA FLS from 9 patients were obtained by seeding 6-well plates in

DMEM containing 10% FBS and incubated in at 37 °C in a humidified 5% CO₂ incubator. After culturing for 12 h, RA FLS were starved in 6 h serum-free DMEM. RA FLS were then treated with recombinant human IL-1 β (10ng/ml) (R&D Systems, Minneapolis, MN, USA).

2.4. Next-Generation Sequencing (NGS) :

Total RNA (including lncRNA) was extracted from the 5 RA FLS and 4 RA FLS with IL-1 β . High-quality RNA with the ratio of the absorbance at 260 and 280 nm (A₂₆₀/A₂₈₀ ratio) of 1.8-2 was measured by a NanoDrop spectrophotometer (Thermo Fisher Scientific, MA, USA). The RNA purity was confirmed by electrophoresis of 18S and 28S ribosomal RNA bands on a 1% agarose gel. Finally, the RNA integrity number (> 7.0) was measured using an Agilent 2100 bioanalyzer (Agilent, Palo Alto, CA, USA). Reads were aligned to the *Homo sapiens* reference genome (hg19; <http://genome.uscs.edu>) using Tophat v2.0.13 and Bowtie v2.2.3 (17). NGS was performed through Macrogen Inc. (Seoul, Korea).

2.5. NGS Data Analysis:

NGS data were log-transformed, normalized, and quantile normalized. The NGS data was analyzed to identify any significant lncRNAs expressed between RA FLS and RA FLS treated with IL-1 β . To visualize differentially expressed transcriptomes, volcano plots were generated by classification according to log₂ fold changes (x-axis) and statistical significance (y-axis). Common expression patterns of transcripts differentially expressed in RA FLS and RA FLS treated with IL-1 β were shown using hierarchical cluster analysis. LncRNAs

were selected when the absolute value of the fold change was more than 2 and $p < 0.05$. Potential functions of lncRNA, including biological processes and cellular components, in RA, were identified through gene ontology (GO) analysis.

2.6. Quantitative Real-Time PCR Analysis (RT-qPCR) :

Total RNA (including lncRNA) was isolated from RA FLS ($n = 9$) using the RNeasy Micro Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Reverse transcription reactions were performed using a high-dose cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The expression levels of lncRNA were measured by real-time polymerase chain reaction (RT-qPCR) using the CFX Connect Real-Time PCR System (Bio-Rad, Hercules, CA, USA). The primer sequences used for RT-qPCR were synthesized by Bionics (Seoul, Korea), and are summarized in Table 1. For each PCR reaction, a total volume of 20 μ L was used, composed of 2 μ L of template cDNA (10 ng/ μ L), 1 μ L of forward primer, 1 μ L of reverse primer, 10 μ L of $2 \times$ SYBR Green Master Mix (Toyobo, Osaka, Japan), and DNase/RNase-free distilled water. Each experiment was performed in duplicate. As an endogenous control, β -actin expression was used as the $2^{-\Delta\Delta C_t}$ method to normalize lncRNA expression.

2.7. Western Blot Analysis:

FLS were treated with 20 μ M IMD-0560 (MedChemExpress, Monmouth Junction, NJ, USA) and MG-132 (Enzo Life Sciences, Madison Avenue, NY, USA) for 2 h, then stimulated with 10 ng/ml of IL-1 β . The cells

were then treated with lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA) containing a phosphatase inhibitor (Roche Diagnostics GmbH, Mannheim, Germany) and protease inhibitors (Sigma-Aldrich, Steinheim, Germany). The total protein was extracted from the cells, whose concentration was measured by the bicinchoninic acid Protein Assay (BCA) kit (Pierce; Thermo Fisher Scientific, Inc., Rockford, IL, USA). The same amount of protein was separated on a 10% SDS-polyacrylamide gel before transferring to an Immun-Blot PVDF Membrane (Bio-rad, Hercules, CA, USA). The primary antibody used was I κ B α or phospho-I κ B α (1 : 2000) (Abcam, MA, USA). Anti-rabbit IgG, HRP-linked Antibody (1 : 2000) (Cell Signaling Technology, MA, USA) was used as the secondary antibody. The proteins were visualized using Fusion Solo (Vilber Lourmat, Marne-la-Vallée, France).

2.8. Statistical Analysis:

The differential lncRNA expression between RA FLS and RA FLS stimulated with IL-1 β was analyzed using Student' s *t*-test. It was considered significant when the p-value was less than 0.05. Statistical analysis was performed using SPSS version 20.0 software for Windows (IBM Corp., Armonk, NY, USA). Data are expressed as the mean $\pm$ standard deviation (SD).

Table 1. Primer Sequences for Validation of LncRNAs

LncRNA	Up-stream primer sequence (5 to 3')	Down-stream primer sequence (5 to 3')
NR_046035	GCGCAGCAGTCAGTGAGTC	TGCAGGGTCTGCAAGCAC
NR_027783	GGCGGGGAGGTAATAAAAG	CAATCCACGGGTCATAGGTAA
NR_033422	GTTAGTTCAGAGAGACGGGATTT	GGATTGCTGCATCAACACCT
NR_003133	GTGCTAGAAGCCACTCCTCA	GGCCTGTCATGTGGATCTCT
NR_049759	CATGTCGTCTGGTCCCTGTT	CTTCCTGTCCCTTCACGGAG

LncRNA: long non-coding RNA.

3. Results

3.1. NGS Results:

By comparing RA FLS (n = 5) and RA treated FLS with IL-1 β (n = 4), approximately 4,360 lncRNA were identified using NGS. Distinct lncRNA expression patterns were confirmed by hierarchical cluster analysis. Differentially expressed lncRNAs between RA FLS and RA FLS treated with IL-1 β are shown as a volcano plot (Figure 1).

3.2. Expression Levels of LncRNAs:

Among the lncRNAs found by NGS analysis, those with a |fold change| ≥ 2 and a $p < 0.05$ were selected. The NGS results showed that 30 upregulated and 15 downregulated lncRNAs were expressed differentially between RA FLS and RA FLS treated with IL-1 β (Figure 2).

3.3. Functional Analysis:

GO analysis was used to confirm the function of the differentially expressed lncRNAs identified through NGS. The lncRNAs differentially expressed between RA FLS and RA FLS treated with IL-1 β were related to biological processes, including system development, anatomical structure morphogenesis, negative regulation of blood coagulation. Also, these lncRNAs were related to cellular components including the lysosomal membrane, vacuolar membrane, and vacuolar part (Figure 3).

3.4. NGS Data Validation:

From the 45 lncRNAs identified by NGS, I selected 5 upregulated lncRNAs (NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759) for RT-qPCR validation using RA FLS (n = 9) and RA FLS treated with IL-1 β (n = 9). The 5 lncRNAs were selected based on their p-value, fold change, and primer specificities. RT-qPCR was used for the validation of 5 differentially expressed lncRNAs in RA FLS and RA FLS treated with IL-1 β . As in the NGS result, the RT-qPCR results revealed that 5 candidate lncRNAs (NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759) (p < 0.05) were significantly upregulated in RA FLS treated with IL-1 β compared to RA FLS (Figure 4).

3.5. Degradation and Phosphorylation of I κ B α :

I confirmed the changes in the expression of the candidate lncRNAs in RA FLS when the NF- κ B pathway was inhibited by IMD-0560. IMD-0560 suppressed IL-1 β induced phospho-I κ B α activity in a dose-dependent manner, as confirmed by western blot analysis. Also, 20 μ M MG-132 blocked I κ B α degradation (Figure 5).

3.6. IMD-0560 Treatment Reduced the Expression of IL-1 β Induced lncRNAs:

I confirmed the expression of the upregulated 5 lncRNAs (NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759) in RA FLS (n = 9) compared to RA FLS treated with IL-1 β (n = 9) using RT-qPCR.

NR_046035, NR_027783, NR_033422, and NR_003133 ($p < 0.05$) were significantly downregulated in RA FLS by IMD-0560 compared to RA FLS stimulated with IL-1 β . NR_049759 tended to decrease but had no statistical significance in RA FLS treated with IL-1 β , compared to RA FLS treated by IMD-0560 and IL-1 β . This data showed that the inhibition of I κ B α phosphorylation also reduced the expression of 4 candidate lncRNAs (NR_046035, NR_027783, NR_033422, and NR_003133) (Figure 6).

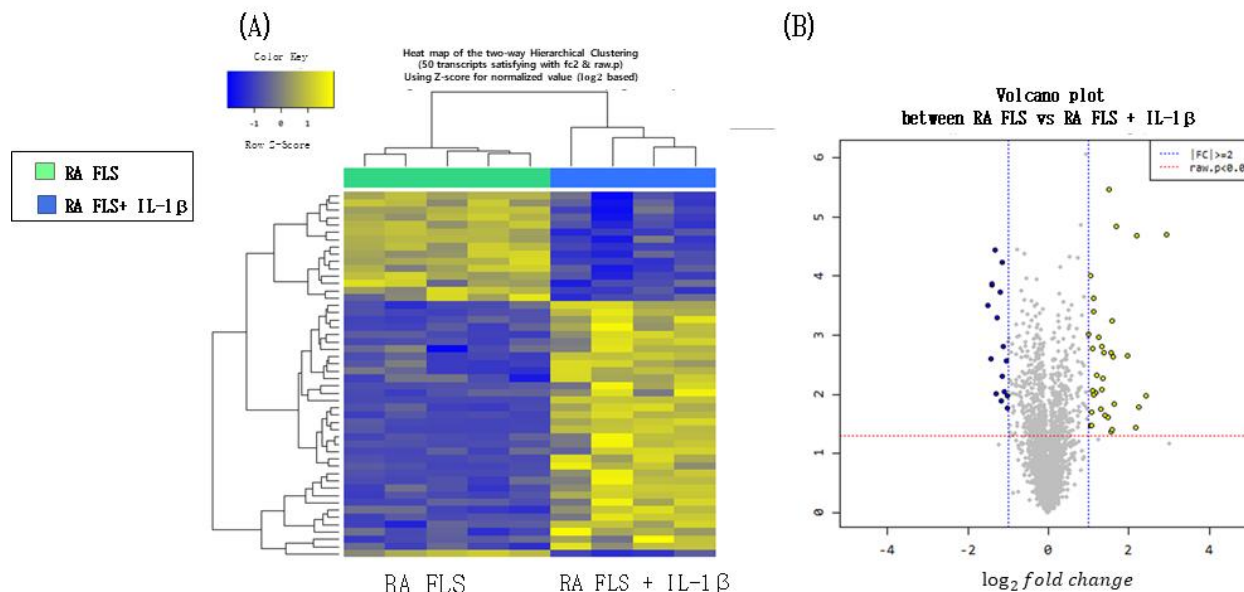


Figure 1. Hierarchical clustering heatmap and volcano plot analysis of differentially expressed lncRNAs. (A) Heatmap of differentially expressed lncRNAs. Total RNA harvested from RA FLSs (n = 5) and RA FLS + IL-1 β (n = 4) was screened by NGS. (B) Volcano plot shows differentially expressed lncRNAs between RA FLSs and RA FLS + IL-1 β . FLS: fibroblast like synoviocytes; lncRNA: long non-coding RNA; RA: rheumatoid arthritis.

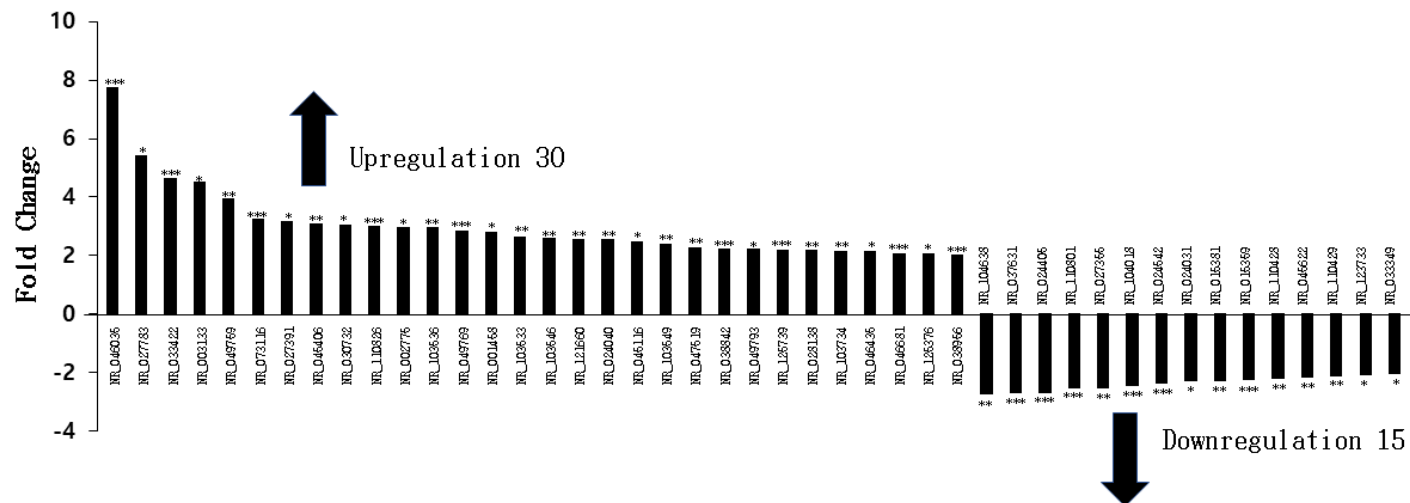
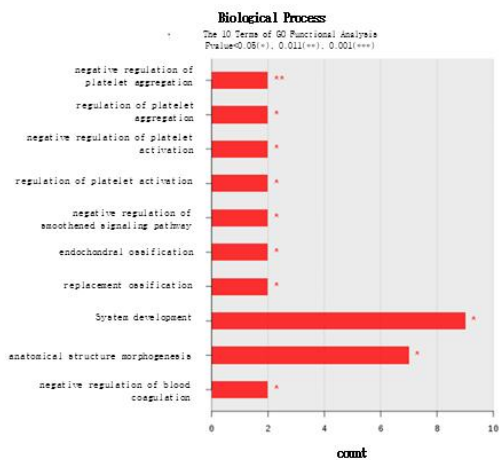


Figure 2. Comparison of lncRNA expression profiles between RA FLS and RA FLS treated with IL-1 β . Upregulated and downregulated lncRNAs in RA FLS were compared with RA FLS treated with IL-1 β . * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. FLS: fibroblast like synoviocytes; lncRNA: long non-coding RNA; RA: rheumatoid arthritis.

(A)



(B)

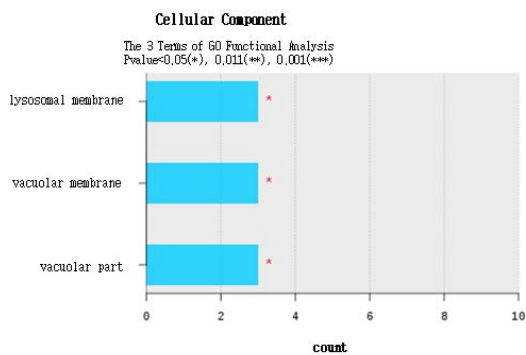


Figure 3. GO functional analysis of GO terms for differentially expressed lncRNAs. The top 200 GO function terms. (A) Biological process and (B) cellular components for the co-expression genes of differentially expressed lncRNAs between FLS treated with IL-1 β (10 ng/ml) and RA FLS. FLS: fibroblast like synoviocytes; LncRNA: long non-coding RNA; RA: rheumatoid arthritis.

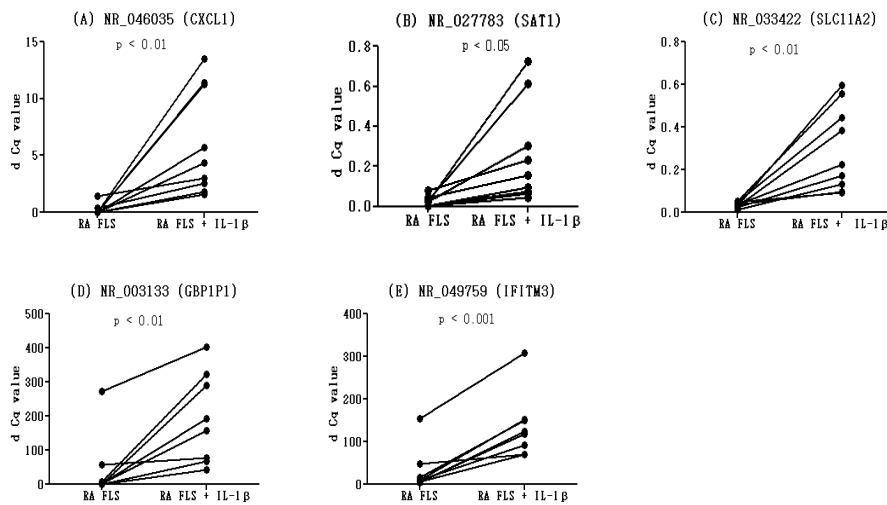


Figure 4. Validation of upregulated lncRNAs using RT-qPCR. Correlation between NR_046035 (A), NR_027783 (B), NR_033422 (C), NR_003133 (D), and NR_049759 (E) in RA FLS compared with RA FLS treated with IL-1 β . paired two-tailed *t*-test. FLS: fibroblast like synoviocytes; lncRNA: long non-coding RNA; RA: rheumatoid arthritis.

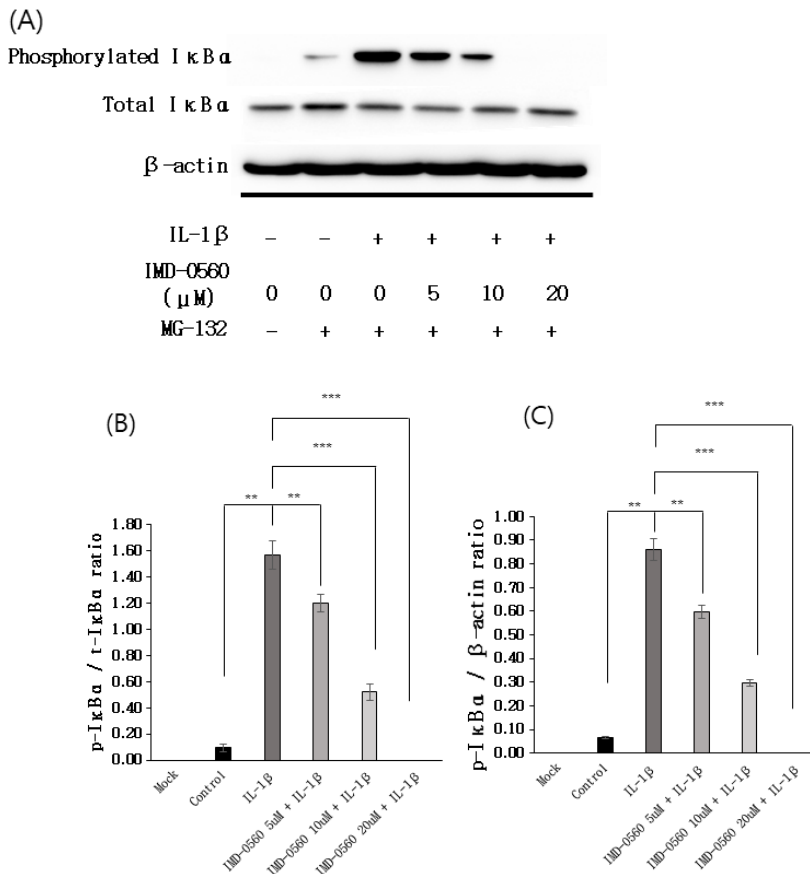


Figure 5. Effect of IMD-0560 on IL-1 β induced phosphorylation of I κ B α . FLS were pre-treated with 20 μ M MG-132. After treatment with or without IMD-0560 (20 μ M) for 2 h, RA FLS were stimulated with IL-1 β (10 ng/ml) for 30 min. Phosphorylation of I κ B α induced by IL-1 β was analyzed using western blot analysis. Data are presented as the mean $\pm$ standard error of the mean from 3 separate experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. FLS: fibroblast like synoviocytes; LncRNA: long non-coding RNA; RA: rheumatoid arthritis.

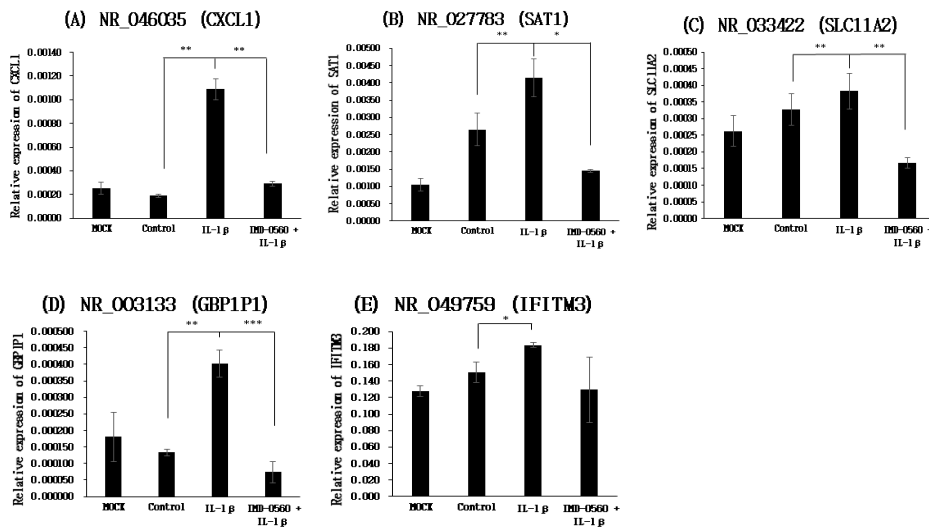


Figure 6. LncRNAs upregulated by IL-1 β are downregulated by inhibiting I κ B α phosphorylation. FLS were pre-treated with 20 μ M MG-132. After treatment with or without IMD-0560 (10 μ M) for 2 h, RA FLS were stimulated with IL-1 β (10 ng/ml) for 1 h (A, B), 2 h (C, E), and 6 h (D). The relative expression induced by IL-1 β was analyzed by using real-time RT-PCR and the $2^{-\Delta\Delta C_t}$ method. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

FLS: fibroblast like synoviocytes; LncRNA: long non-coding RNA; RA: rheumatoid arthritis.

4. Discussion

RA is a chronic inflammatory autoimmune disease involving many inflammatory cytokines. Many cytokines are known to play a fundamental role in the process of inflammation, joint destruction, and various disease (18). Among the various pro-inflammatory cytokines involved in the development of RA, IL-1 is known to be a major factor concerned inflammation in RA. IL-1 induces cytokine production, MMP release, catabolism, and cytokine production in vitro (19). IL-1 feeds on cytokines, resulting in the production of various proteins, including IL-1 receptor antagonists (IL-1Ra) via diverse signaling pathways (20-22). Many researchers have suggested that IL-1 is an important cytokine involved in inflammation and play a key role in the pathogenesis of RA (23,24). IL-1 β is an agonist molecule that can bind to IL-1 receptors and affect the function of most cell types. IL-1 β causes aggravation of the disease stimulates the absorption of cartilage and bone. In addition, there is a study that inhibits the synthesis of joint collagen and proteoglycans in experimental models of arthritis (25). Targeting the IL-1 in rodent arthritis models was found to be useful in reducing especially joint injury (26,27). One study revealed that serum levels of IL-1 β in RA patients were correlated with BMI (28). The reaction of the IL-1 receptor leads to NF-kB signaling and activation of the p38 mitosis-activated protein kinase pathway, leading to the expression of the IL-1 target gene. Thus, blockade of IL-1 β is an effective treatment option (29-31). Most intracellular components mediate responses to cytokines, and many forms of cytotoxic stress (32). Among them, IL-1 is a representative signaling pathway involved in RA

via the NF- κ B signaling pathway. Importantly, NF- κ B is involved in RA and the regulation of inflammatory cytokine production. Pro-inflammatory cytokines are well known to be under the control of the NF- κ B transcription factor (8). As such, this study attempted to confirm the changes in lncRNA by inhibiting NF- κ B. NF- κ B is a dimer transcription factor of the Rel family and is known to play an important role in various inflammatory processes. When IKK activation by IL-1, NF- κ B is activated (33-35). Degradation of I κ B α results in the release of p50 and p65 NF- κ B subunits. This result allows nuclear translocation, a key step in NF- κ B activation (36). Some anti-rheumatic drugs currently being used clinically are able to inhibit the activation of NF- κ B pathway (37). This supports the notion that NF- κ B pathway is involved in the development of RA. NF- κ B inhibitors include IKK complex inhibitors (38), I κ B α degradation inhibitors (39), NF- κ B nuclear translocation inhibitors (40), p65 acetylation inhibitors (41), NF- κ B transactivation inhibitors (42), and antioxidants (43). NF- κ B activation has been reported to require phosphorylation and subsequent degradation of I κ B α , and recent evidence has suggested that I κ B α phosphorylation is regulated by IKK β (44). Therefore, I selected IMD-0560, which is known to inhibit IKK in FLS, for use in this study (45). In one study, using anakinra, the recombinant human IL-1Ra in active RA patients was used to improve the signs and symptoms of RA. The efficacy of anakinra shows that IL-1 plays a key role in RA activity as well as the pathogenesis of RA (46). Consistent with these results, patients with active RA also showed the highest levels of synovial fluid as well as increased plasma levels of IL-1 β (47). Patients with the lowest levels of IL-1 β and IL-1Ra have also been found to have the most severe types of Lyme arthritis (48). Moreover, one study has shown that animals

overexpressing IL-1Ra are protected from endotoxins and can die. (20). Overexpressed IL-1 β contributes to the onset of T2D, causing β -cell impaired functioning and apoptosis, consequently reducing insulin production. In addition, IL-1 β induce a unique mitochondrial apoptosis pathway in β cells and can inhibit glucose-stimulated insulin secretion (49). However, IL-1 β related lncRNA studies in RA remain scarce.

In this study, RA FLS were stimulated by IL-1 β , which one of the important factors involved in the pathogenesis of RA. I confirmed that lncRNA expression in the 5 RA FLS and 4 IL-1 β -treated RA FLS samples by NGS analysis and found that 45 lncRNAs were differentially expressed between RA FLS and IL-1 β -treated RA FLS. As such, I validated the NGS results for the upregulated 5 candidate lncRNAs differentially expressed using RT-qPCR for 9 RA FLS and IL-1 β -treated RA FLS samples. The lncRNAs NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759 were found to be significantly expressed between RA FLS and IL-1 β -treated RA FLS, which was consistent with the NGS results.

Several studies have been reported on these lncRNAs in other diseases. Jin et al. (50) reported that NR_046035 was involved in immune and inflammatory responses, providing new targets of the treatment of diabetic nephropathy. This lncRNA is markedly increased in the regulation of protein metabolic processes, and is mostly rich in immune-related processes, such as immune responses, inflammatory responses, and innate immune responses. Song et al. (51) demonstrated that proteins encoded by NR_027783 are members of the acetyltransferase family and rate-limiting enzymes and is involved in polyamine metabolism. Moreover, NR_027783 is an important RNA molecule in chronic thromboembolic pulmonary hypertension. Dong et

al. (52) reported that NR_049759 is the transcript variant 2 of the coding gene IFITM3, which is upregulated in the mucosa of DSS-induced mice. It has also been reported to have statistically significant changes in the animal model of Crohn's disease. Recently, several studies have demonstrated that various lncRNAs may be involved in autoimmune diseases, including RA. The most common approaches to identifying lncRNAs are RT-qPCR and microarray (53). Target cells range from peripheral blood cells to fibroblasts, such as FLS. Recently, the important role of lncRNA in the pathogenesis of RA has reported through various studies. In 2017, Zhang et al. (54) examined the regulation of Hox transcript antisense intergenic RNA (HOTAIR) in RA mice and LPS-treated chondrocytes. Transfection with a recombinant lentivirus was used to find that HOTAIR was overexpressed in chondrocytes. Overexpression of HOTAIR resulted in the downregulation of IL-1 β by inhibiting NF- κ B activation in LPS-treated chondrocytes and inhibiting p65 in the nucleus. These results suggested that HOTAIR involved in the pathogenesis pathway of RA (55). In 2015, Lu et al. (56) confirmed that LOC100652951 and LOC100506036 expression in T cells of the control group was lower than in the RA group. RA patients treated with biologics showed low expression levels of LOC100652951, and female RA patients were found to have low LOC100506036 expression levels by multivariate analysis. Thus, treatment with biological agents can lower the expression of LOC100652951 in RA T cells. In addition, LOC100506036 may contribute to the inflammation in RA by regulating the expression of sphingomyelin phosphodiesterase 1. Mo et al. (57) reported that lncRNA GAPLINC expressed in FLSs in the RA group was greater than in the health control. LncRNA GAPLINC inhibition in RA FLS cells showed significantly reduced cell proliferation, infiltration, migration,

and pro-inflammatory cytokine production. These results suggest that some miRNAs and messenger RNAs (mRNAs) may interact with GAPLINC. Results confirmed with GAPLINC silencing showed increased expression of miR-382-5p and miR-575 after GAPLINC silencing. Thus, GAPLINC promotes RA FLS tumor-like behavior in a miR-382-5p-dependent and miR-575-dependent manner. Although the number of studies of RA-related lncRNAs is increasing, this study is the first to use NGS for FLS. Firstly, I investigated the effect of IMD-0560 on the NF- κ B signaling pathway in RA FLS. I observed that IMD-0560 inhibits the IL-1 β induced I κ B phosphorylation activity of NF- κ B in RA FLS. Next, after treatment with IMD-0560, I confirmed that the expression of 4 lncRNAs (NR_046035, NR_027783, NR_033422, and NR_003133) induced by IL-1 β was reduced. As a result, the lncRNAs induced by IL-1 β are likely to be involved in the NF- κ B signaling pathway. Further studies and validation using a larger number of FLS samples may help to understand the mechanisms involved in RA for the 5 candidate lncRNAs in the clinical diagnosis of this disorder.

In conclusion, the results presented here confirmed that the expression of lncRNA was changed by IL-1 β treatment in RA FLS using NGS. When the NF- κ B pathway was inhibited, the expression of candidate lncRNAs was reduced. Thus, my data suggest that lncRNA may be involved in the development of RA via the NF- κ B signaling pathway.

5. Summary

LncRNA has recently emerged as an important biological regulator involved in various diseases, but the role of lncRNA in the pathogenesis of RA is unknown. In this study, the expression of lncRNAs in both RA FLS and IL-1 β -treated RA FLS was analyzed using NGS. I identified 45 lncRNAs that were differentially expressed between RA and IL-1 β -treated RA FLS. Next, I validated the NGS results for NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759, which were significantly upregulated in IL-1 β -treated RA FLS compared to RA FLS. Among these validated 5 lncRNAs, the expression of 4 lncRNAs (NR_046035, NR_027783, NR_033422, and NR_003133) was decreased after treatment with IMD-0560. This study is the first to identify the lncRNAs induced by IL-1 β in RA FLS using NGS. The expression of candidate lncRNAs decreased as the NF- κ B pathway was inhibited. Therefore, my data suggest that these lncRNAs may be involved in the development of RA via the NF- κ B signaling pathway.

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Expression of Long Non-Coding RNAs
Induced by IL-1 β is Changed
by Inhibiting I κ B α Phosphorylation

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(Abstract)

Long non-coding RNAs (lncRNAs) have recently emerged as important biological regulators. However, the role of lncRNAs in the pathogenesis of rheumatoid arthritis (RA) remains unknown. This study focuses on lncRNAs influenced by interleukin (IL) -1 β , a key mediator of the pathogenesis of RA. The next-generation sequencing (NGS) data were analyzed to identify differentially expressed lncRNAs between 5 unstimulated RA fibroblast-like synoviocytes (RA FLS) and 4 IL-1 β -stimulated RA FLS. The NGS data were validated by real-time PCR (RT-qPCR) using 9 unstimulated RA FLS and IL-1 β -stimulated RA FLS. NGS analysis revealed 30 upregulated lncRNAs and 15 downregulated lncRNAs in IL-1 β -treated RA FLS, compared with

unstimulated RA FLS. The top 5 lncRNAs (NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759) were selected among the 30 lncRNAs upregulated based on the fold-changes in their expression, using a p-value cut-off. IMD-0560, I κ B kinase β inhibitor, blocked phosphorylation of I κ B α and also inhibited overexpression of lncRNAs induced by IL-1 β in RA FLS. The expression of NR_046035, NR_027783, NR_033422, and NR_003133 was regulated by the inhibition of NF- κ B activation. In this study, I found out lncRNAs influenced by IL-1 β using NGS and my data suggest that these lncRNAs may be involved in the pathogenesis of RA via NF- κ B pathway.

I κ B α 인산화를 억제함으로 발생하는 IL-1 β 에 의해 유도된 긴 비번역 RNAs의 발현 변화

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(초록)

긴 비번역 RNA(lncRNA)는 최근 중요한 생물학적 조절제로 등장했으며 lncRNA의 비정상적인 발현은 암을 포함한 다양한 질병에서 보고되었다. 그러나 류마티스관절염의 발병 기전에서 lncRNA의 역할은 알려져 있지 않다. 따라서, 나는 류마티스관절염의 병인에서 주요 매개체 중 하나인 인터류킨 (IL) -1 β 에 의해 발현이 되는 lncRNAs를 연구하고, IL-1 β 에 의해 유도되는 것으로 알려진 nuclear factor-kappa B(NF-kB) 활성화의 조절이 이들 lncRNAs의 발현에 변화를 주는지 확인하였다. 차세대 시퀀싱(NGS) 데이터를 분석하여 5 개의 자극되지 않은 류마티스관절염 활막세포와 4 개의 IL-1 β 으로 자극된 류마티스관절염 활막세포 사이에서 차등적으로 발현된 lncRNAs를 확인하였다. NGS 데이터에서 상향조절 되는 상위 5 개 후보 lncRNAs의 발현 수준은 9 개의 자극되지 않은 류마티스관절염 활막세포 및 IL-1 β 으로 자극된 류마티스관절염 활막세포를 사용하여 실시간 유전자 분

석 장치(RT-qPCR)에 의해 검증되었다. IMD-0560을 NF- κ B 활성화의 조절에
 사용하였다. NGS 분석은 자극되지 않은 류마티스관절염 활막세포와 비교하
 여 IL-1 β 처리된 류마티스관절염 활막세포에서 상향 조절된 30 lncRNAs
 및 하향 조절된 15 lncRNAs를 밝혀냈다. 통계적 유의성을 가졌으며 배수
 변화에 기초하여 류마티스관절염 활막세포에서 IL-1 β 에 의해 상향 조절된
 30 개의 lncRNAs 중에서 상위 5 개의 lncRNAs를 선택하였다. NR_046035,
 NR_027783, NR_033422, NR_003133 및 NR_049759를 포함하는 상향 조절된
 lncRNAs는 RT-qPCR에 의해 확인되었다. IMD-0560은 류마티스관절염 활막세
 포에서 IL-1 β 에 의해 유도된 I κ B α 의 인산화를 억제하였다. IL-1 β 에 의
 해 유도된 lncRNAs의 과발현은 또한 류마티스관절염 활막세포에서
 IMD-0560에 의해 억제되었다. IL-1 β 가 류마티스관절염 활막세포에서
 NR_046035, NR_027783, NR_033422, NR_003133 및 NR_049759의 발현을 증가
 시키는 것으로 나타났다. 또한, 이들 lncRNAs의 발현은 NF- κ B 활성화의 억
 제에 의해 조절되었다. 본 연구는 처음으로 NGS를 사용하여 류마티스관절
 염 활막세포에서 IL-1 β 에 의해 영향을 받는 lncRNAs를 발견하였으며, 이
 들 후보 lncRNAs가 NF- κ B 신호 전달 경로를 통해 류마티스관절염의 발병에
 관여할 수 있음을 시사한다.