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Enhanced Effect of Hyaluronan and Elastin Synthesis in Fibroblasts Through Lipopolysaccharide-activated Macrophages

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Abstract. *Background/Aim: The functions of macrophages change in response to environmental factors such as lipopolysaccharide (LPS). LPS derived from Pantoea agglomerans (LPSp) is involved in macrophage activation and tissue repair when administered dermally. LPSp-activated macrophages may be useful for restoring and maintaining homeostasis of the skin. Materials and Methods: Phorbol myristate acetate-treated human monocytes (THP-1 cells) were activated with LPSp. The medium of LPSp-activated THP-1 cells was added to normal human dermal fibroblasts (NHDF cells). After 24 h, the expression of hyaluronan (HA) synthase (HAS)2, hyaluronidase (HYAL)1, and tropoelastin in NHDF cells was analyzed using quantitative real-time PCR. Results: The expression of HAS2 and tropoelastin was significantly increased, but that of HYAL1 was significantly decreased. It was demonstrated that the abilities of HA and elastin synthesis in NHDF cells increased through LPSp-activated THP-1 cells. Conclusion: LPSp-activated macrophages may be useful for enhancing the abilities of HA and elastin synthesis in fibroblasts, subsequently improving dysfunction and reducing various age-related disorders.*

Monocytes migrate into tissues, and differentiate into macrophages with tissue-specific properties depending on the microenvironment. Macrophages recognize and eliminate foreign substances and play an important role in maintaining homeostasis (1-4). Macrophages that have accumulated in tumor tissues or adipose tissues in people with obesity will interact with cancer cells or adipocytes, resulting in increased inflammatory cytokine production and incurring inflammatory changes within the tissues. As a result, these macrophages are considered to induce chronic inflammation, which is involved in the development of malignant tumors and lifestyle-related diseases (5-7). Therefore, it is thought that the function of macrophages can change in response to environmental factors.

Lipopolysaccharide (LPS) is an extracellular membrane component of gram-negative bacteria. Macrophages activated with LPS induce the expression of inflammatory cytokines (8). In contrast, it has also been reported that the increased expression of inflammatory cytokines in monocytes/macrophages by co-culture with cancer cells or adipocytes can be suppressed by pretreatment with low-dose LPS derived from *E coli* (LPSe) (9-12). Thus, it is suggested that the function of macrophages can change from inflammatory to anti-inflammatory in response to environmental factors such as low-dose LPSe. Moreover, LPS derived from *Pantoea agglomerans* (LPSp) has been demonstrated to have tissue repair effects and be nontoxic when administered orally and dermally (13). The function of LPS is reported to be closer to that of an exohormone than an endotoxin (14). Therefore, LPSp-activated macrophages may be harmless and useful for restoring and maintaining homeostasis of the intestinal tract and skin.

In the dermis layer of the skin, macrophages and fibroblasts are thought to interact to maintain homeostasis. Fibroblasts produce extracellular matrix components such as hyaluronan (HA), elastin, and collagen. HA is a high molecular weight polysaccharide that is present at high concentrations in skin tissue (15). HA can retain water and is viscous, and is essential for maintaining tissue structure. HA is also involved in cell proliferation, differentiation, development, and wound healing (16-18). Elastin is an insoluble protein that is widely distributed throughout skin tissue (19). Elastin is one of the main components of elastic fibers and is involved in maintaining the structure and physical properties (strength and viscoelasticity) of the skin. Therefore, HA and elastin,

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Key Words: Monocyte, macrophage, fibroblast, hyaluronan, elastin, lipopolysaccharide.

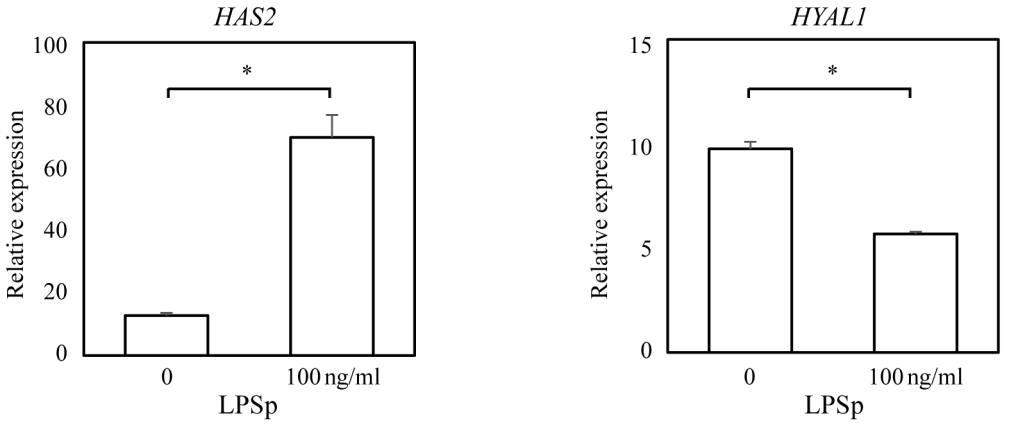

Figure 1. *The effect of LPSp-activated macrophages on the gene expression of HAS2 and HYAL1 in NHDF cells. The mRNA expression of HAS2 and HYAL1 in NHDF cells was analyzed using quantitative real-time PCR. Relative quantification was performed by normalizing the target expression to that of GAPDH. *p<0.01.*

produced by fibroblasts, both play important roles in maintaining the structure and function of the skin.

The amount of HA and elastin synthesis by fibroblasts decreases with age, in turn reducing the function of skin tissue (20, 21). Therefore, increasing the amount of HA and elastin synthesis by fibroblasts in skin tissue may improve dysfunction and reduce the effects of various age-related disorders such as osteoarthritis. However, the effect of LPSp-activated macrophages on fibroblasts is not clear. In this study, we investigated changes in HA and elastin synthesis by fibroblasts through LPSp-activated macrophages.

Materials and Methods

Cells. Human monocytes (THP-1 cells) were obtained from American Type Culture Collection, and normal human dermal fibroblasts (NHDF cells) were obtained from Kurabo (Kurabo Industries, Ltd., Osaka, Japan). THP-1 cells in RPMI 1640 medium (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) containing 10% fetal calf serum supplemented with 100 units/ml each of penicillin and streptomycin (FUJIFILM Wako Pure Chemical Corporation) and NHDF cells in DMEM medium (FUJIFILM Wako Pure Chemical Corporation) containing 10% fetal calf serum supplemented with 100 units/ml each of penicillin and streptomycin (FUJIFILM Wako Pure Chemical Corporation) were incubated in 5% CO_2 atmosphere at 37˚C.

Addition of LPSp-activated THP-1 cells medium. THP-1 cells were seeded at a density of 5×10^5 cells/ml in a dish. THP-1 cells were treated with 5 ng/ml phorbol myristate acetate (FUJIFILM Wako Pure Chemical Corporation) for 48 h and activated with 100 ng/ml LPSp (Macrophi Inc., Kagawa, Japan). THP-1 cells medium was collected at 24 h after activation with LPSp. NHDF cells were seeded at a density of 3×10^5 cells in a dish. After 24 h, the NHDF cells medium was removed, and LPSp-activated THP-1 cells medium and DMEM medium were added in equal amounts to NHDF cells. Then, the cells were incubated in 5% CO_2 atmosphere at 37˚C for 24 h.

RNA extraction. The total RNA from NHDF cells was extracted using TRIzol® Reagent (Invitrogen Corporation, Carlsbad, CA, USA) in accordance with the manufacturer's protocol. RNA was quantified by absorbance at 260 nm. cDNA was synthesized using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Co., Ltd., Osaka, Japan).

Quantitative real-time PCR. Real-time PCR was performed using iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA). PCR conditions were set at 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 s, 60˚C for 30 s and 72˚C for 30 s. Relative quantification was performed by normalizing the target expression to that of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*).

Statistical analysis. Data were analyzed with Student's *t*-test (Excel 2013). Differences between stimulation with and without LPSp were considered statistically significant at $p<0.05$.

Results

Effect of LPSp-activated macrophages on HA synthesis. The balance between HA synthesis and degradation is considered important for the functional expression of HA (22). HA is synthesized by three types of HA synthase (HAS). It is reported that HAS2 is the main contributor to HA synthesis in the skin dermis (23). HA is degraded by hyaluronidase (HYAL). There are several types of HYAL, and HYAL1 is mainly released from fibroblasts (24). We investigated the expression of *HAS2* and *HYAL1* in NHDF cells after treatment with LPSp-activated THP-1 cells medium. There was a significant increase in the expression of *HAS2* in NHDF cells through LPSp (100 ng/ml)-activated THP-1 cells ($p=0.0027$). Moreover, there was a significant decrease in the expression of *HYAL1* in NHDF cells through LPSp (100 ng/ml)-activated THP-1 cells

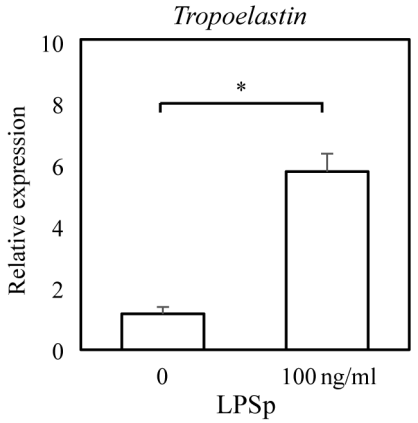

Figure 2. *Effect of LPSp-activated macrophages on the gene expression of tropoelastin in NHDF cells. The mRNA expression of tropoelastin in NHDF cells was analyzed using quantitative real-time PCR. Relative quantification was performed by normalizing the target expression to that of GAPDH. *p<0.01.*

(*p*=0.0013) (Figure 1). It was demonstrated that the ability of HA synthesis in NHDF cells increased by LPSp-activated THP-1 cells.

Effect of LPSp-activated macrophages on elastin synthesis. Elastin is secreted from fibroblasts in the skin dermis as tropoelastin, a soluble precursor of mature elastin (19). Elastin fiber has a very long turnover time (25, 26). We investigated the effect of LPSp-activated THP-1 cells on the expression of *tropoelastin* in NHDF cells. We found that the expression of *tropoelastin* in NHDF cells was significantly increased by LPSp (100 ng/ml)-activated THP-1 cells (*p*=0.0005) (Figure 2). It was demonstrated that the ability of elastin synthesis in NHDF cells increased through LPSp-activated THP-1 cells.

Effect of LPSp-activated macrophages on collagen synthesis. Type I collagen is most abundant in vertebrates and comprises α1 chain (*COL1A1*) and α2 chain (*COL1A2*) (27). Moreover, it is reported that the expression of *HSP47* always correlates with those of *COL1A1* and *COL1A2* (28). In addition, HSP47-knockout mice specifically have abnormal type I collagen (29). We investigated the expression of *COL1A1*, *COL1A2*, and *HSP47* in NHDF cells through LPSp-activated THP-1 cells. No significant difference was found in the expression of *COL1A1*, *COL1A2*, and *HSP47* in NHDF cells through LPSp (100 ng/ml)-activated THP-1 cells (Figure 3). It was demonstrated that the ability of type I collagen synthesis in NHDF cells did not change by LPSp-activated THP-1 cells.

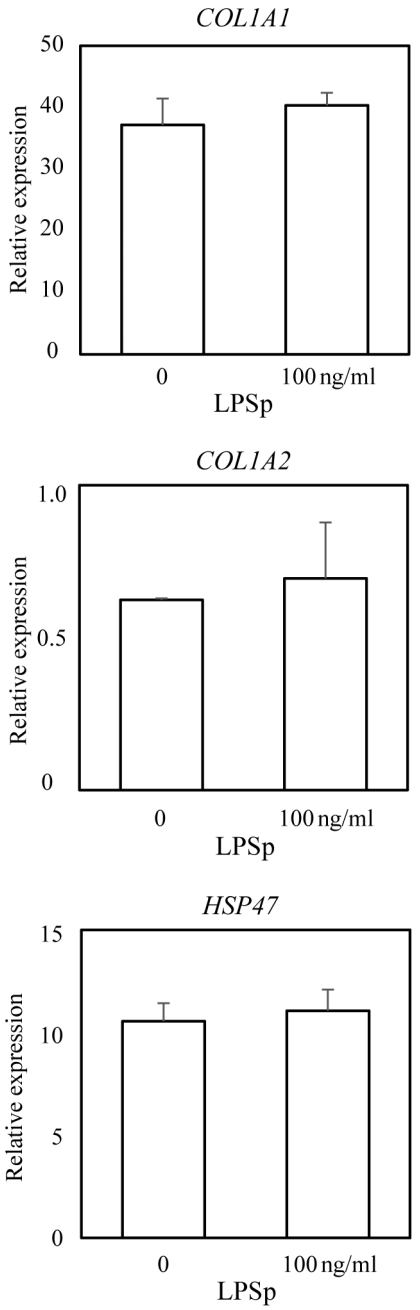

Figure 3. *Effect of LPSp-activated macrophages on the gene expression of COL1A1, COL1A2, and HSP47 in NHDF cells. The mRNA expression of COL1A1, COL1A2, and HSP47 in NHDF cells was analyzed using quantitative real-time PCR. Relative quantification was performed by normalizing the target expression to that of GAPDH.*

Discussion

The skin is an organ that is constantly in contact with the external environment. Therefore, many immune cells such as macrophages are present in skin tissue. It is thought that

macrophages in the skin maintain homeostasis by interacting with cells such as fibroblasts. Fibroblasts produce extracellular matrix such as HA, elastin, and collagen. HA is essential for maintaining tissue structure but is also known to be involved in cell proliferation, differentiation, development, and wound healing (16-18). Approximately 50% of HA in the human body is found in skin tissue and about one-third of the HA in skin tissue is replaced daily (24, 25). Therefore, it is thought that the balance between HA synthesis and degradation is important for the functional expression of HA (22). The results of our study demonstrated that the expression of *HAS2* can be significantly increased and that of *HYAL1* degrading HA can be significantly decreased in NHDF cells through LPSp-activated macrophages (Figure 1). Thus, LPSp-activated macrophages may be useful for enhancing the ability of HA synthesis in fibroblasts, subsequently improving dysfunction and reducing various age-related disorders such as osteoarthritis.

Elastin is one of the main components of elastic fibers. Elastin is involved in maintaining the structure, and physical properties of the skin (19). The results of our study demonstrated that the expression of elastin in NHDF cells can be significantly increased through LPSp-activated macrophages (Figure 2). In skin, it is known that the turnover time of elastin fibers is very long. Once elastin fiber becomes depleted, it is known to be very difficult to reconstruct due to the long process of regeneration (25, 26). Therefore, it is suggested that the enhanced effect of elastin synthesis in fibroblasts through LPSp-activated macrophages may be useful in tissue repair by remodeling elastin.

Collagen is a major component of the extracellular matrix in skin tissue. Collagen is a protein and there are about 29 types in vertebrates. Type I collagen is most abundant in vertebrates and is involved in elasticity and strength (27). It is known that collagen has a slower synthesis and degradation rate than other proteins. The results demonstrated that the ability of type I collagen synthesis in NHDF cells did not change by macrophages activated with LPSp for 24 h (Figure 3). It is possible that the amount of collagen synthesis in fibroblasts did not increase due to the shorter LPSp activation time (24 h) on macrophages. The amount of collagen synthesis does not change with age, but the amount of collagen degradation increases with age (30). This could explain why no change in the amount of collagen synthesis in fibroblasts may have been observed. Thus, it is necessary to investigate the amount of collagen degradation in fibroblasts induced by LPSp-activated macrophages.

The reduction in HA and elastin synthesis results in a reduction of the function of skin tissue in elderly. It has been reported that oral and transdermal administration of LPSp is nontoxic and has a tissue repair effect (13). The results of our study indicate that LPSp-activated macrophages can enhance HA and elastin synthesis in fibroblasts, and subsequently improve skin function and reduce the effects of various age-related disorders.

Conflicts of Interest

This research was partly funded by Macrophi Inc. ST and CK are employees of Macrophi Inc. The corresponding author had full access to all data of the study and also had final responsibility for the decision to submit for publication.

Authors' Contributions

TH and ST performed experiments. CK and HI designed experiments. All Authors have contributed to data collection and interpretation. TH drafted the manuscript, ST, CK and HI contributed to provide critical revision of the manuscript.

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