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# $\beta$-1,4-Galactan suppresses lipid synthesis in sebaceous gland cells via TLR4

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**Satomi Ayaki[1,2], Tomohiro Mii[2], Kosuke Matsuno[2], Takaaki Tokuyama[2], Takahito Tokuyama[2], Takashi Tokuyama[2], Toru Uyama[1] and Natsuo Ueda[1,†]**

[1]Department of Biochemistry, Kagawa University School of Medicine, Miki, Kagawa 761-0793, Japan; and [2] Yushin Brewer Co., Ltd., Ayagawa, Kagawa 761-2307, Japan

[†]Natsuo Ueda, Department of Biochemistry, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki, Kagawa 761-0793, Japan. Tel.: +81-87-891-2102, Fax: +81-87-891-2105, email: ueda.natsuo@kagawa-u.ac.jp

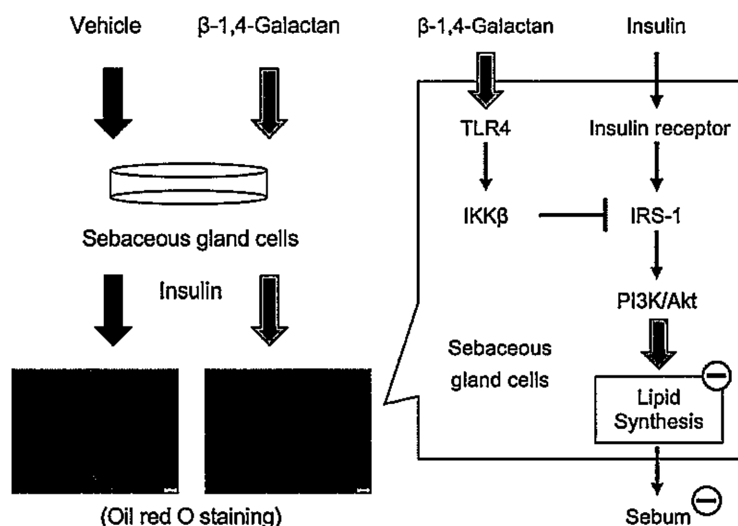
**Sebum is a lipid mixture secreted from sebaceous glands of the skin. The excessive secretion of sebum causes acne vulgaris and seborrheic dermatitis, while its deficiency causes xerosis. Therefore, the appropriate control of sebum secretion is crucially important to keep the skin healthy. In the present study, we evaluated the effects of naturally occurring polysaccharides on lipid biosynthesis in hamster sebaceous gland cells. Among the tested polysaccharides, $\beta$-1,4-galactan, the main chain of type I arabinogalactan, most potently suppressed lipid synthesis in the sebaceous gland cells as analysed by oil red O staining. Toll-like receptor (TLR)4 inhibitors counteracted this suppressive effect and lipopolysaccharide, a TLR4 ligand, mimicked this effect, suggesting the involvement of the TLR4 signalling pathway. In the cells $\beta$-1,4-galactan significantly decreased mRNA levels of lipogenesis-related transcription factors (peroxisome proliferator-activated receptor $\gamma$ and sterol regulatory element-binding protein 1) and enzymes (acetyl-CoA carboxylase and fatty acid synthase) as well as the glucose transporter GLUT4. Furthermore, $\beta$-1,4-galactan increased the production of lactic acid serving as a natural moisturizing factor and enhanced the proliferation of sebaceous gland cells. These results suggest potential of $\beta$-1,4-galactan as a material with therapeutic and cosmetic values for the skin.**

*Keywords:* arabinogalactan; lactic acid; lipopolysaccharide; sebum; skin.

*Abbreviations:* ACC, acetyl-CoA carboxylase; DMEM, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; FAS, fatty acid synthase; FBS, foetal bovine serum; G6PD, glucose-6-phosphate dehydrogenase; IKK$\beta$, I$\kappa$B kinase $\beta$; IRS, insulin receptor substrate; IL, interleukin; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NF$\kappa$B, nuclear factor-kappa B; PPAR$\gamma$, peroxisome proliferator-activated receptor $\gamma$; PI3K, phosphoinositide 3-kinase; PK, pyruvate kinase; qRT-PCR, quantitative real time-PCR; SREBP1c, sterol regulatory element-binding protein 1c; TLR4, Toll-like receptor 4.

The sebaceous gland, which is a skin appendage, opens into the outer root sheath of the upper hair follicle and secretes sebum from the pores to the skin surface through the capillary cavity (*1*). Sebum is composed

Graphical Abstract

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of triglycerides, wax esters, squalene, free fatty acids, cholesterol, sterol esters and diglycerides (2), and the secreted sebum mixes with moisture such as sweat on the skin surface to form a sebum film, which plays physiologically important roles, including barrier function against external substances, bactericidal action and excretion of endogenous substances (3, 4). On the other hand, the dysfunction of sebaceous glands is closely related to the development of skin diseases. For example, the excessive secretion of sebum causes acne vulgaris and seborrheic dermatitis, while the deficiency of sebum causes xerosis (5–7). Therefore, the appropriate control of sebum secretion is crucially important to keep the skin healthy.

Various biomolecules and reagents have been reported to affect the production of sebum. Androgens, insulin, insulin-like growth factor-1 and the agonists of peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) stimulate sebum production, while estrogens, epidermal growth factor (EGF), retinoic acid, niacin and vitamin D suppress sebum production (8–12). In addition, environmental factors such as ultraviolet ray and the change of outside air temperature also affect sebum production (13–15).

Polysaccharides refer to polymers consisting of a large number of monosaccharides such as glucose and mannose linked through glycosidic bonds and exhibit various physical properties and biological functions depending on the type and binding mode of constituent monosaccharides, molecular weight and the forms of main and side chains. For example, arabinoxylan, a plant-derived hemicellulose, has $\beta$-1,4-bonded xylan chains branched with arabinose or 4-*O*-methylglucuronic acid and shows immunostimulatory, antidiabetic and lipolysis-activating effects (16–19). $\beta$-1,3-Glucan, contained in fungi, algae and plants, consists of glucopyranosides polymerized by $\beta$-1,3-bond. This polysaccharide was reported to be involved in immune regulation and wound healing (20–22). Arabinogalactan, widely present in plants, has immunostimulatory and antidiabetic effects and increases beneficial bacteria in intestinal microflora (23–29). Type I arabinogalactan, one of two types, has a $\beta$-1,4-bonded galactan chain branched with arabinobiose or arabinan and is also called pectic arabinogalactan since it binds to the rhamnose units forming a side chain of the pectic polysaccharide, rhamnogalacturonan I. On the other hand, type II has a $\beta$-1,3-bonded galactan chain highly branched with $\beta$-1,6-galactan, arabinose, arabinobiose and arabinan.

Concerning the biological effects on the skin, these bioactive polysaccharides stimulate proliferation, differentiation, collagen production, antioxidation, protection and wound healing on keratinocytes and fibroblasts (21, 30–34). However, their effects on sebaceous gland cells have not yet been reported.

In this study, we evaluated the effects of polysaccharides on sebum production using cultured hamster sebaceous gland cells that resemble human sebaceous gland cells with regard to size, response to androgens and turnover time (35, 36). Our results clearly show the inhibitory effect of $\beta$-1,4-galactan, the main chain of type I arabinogalactan, on sebum production for the first time. We also suggest that the effect is mediated through Toll-like receptor 4 (TLR4), which is known to control the innate immune response by recognizing lipopolysaccharide (LPS) (37).

## Materials and Methods

### *Materials*

L-Arabinose, D-galactose, EGF and insulin were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan). Arabinoxyran (wheat), rhamnogalacturonan I (potato) and $\beta$-1,4-galactan (lupin, average molecular weight 1182 kDa) were from Megazyme (Wicklow, Ireland). Type II arabinogalactan (larch wood) and $\beta$-glucan (black yeast) were from Kanto Chemical (Tokyo, Japan). LPS from *Escherichia coli*, oil red O and phenol red-free Dulbecco's modified Eagle's medium (DMEM)/F12-Ham (1:1) were from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was from Thermo Fisher Scientific (Walthan, MA, USA). TAK-242 was from ChemScene (South Brunswick Township, NJ, USA). TPCA-1 was from Abcam (Cambridge, UK). NucleoSpin RNA was from Macherey-Nagel (Duren, Germany). PrimeScript RT Master Mix and TB Green Premix Ex Taq II were from Takara Bio Inc. (Kusatsu, Japan). Lactate Assay Kit-WST was from Dojindo Laboratories (Kumamoto, Japan). Cytotoxicity Detection Kit$^{PLUS}$ (lactate dehydrogenase, LDH) and Cell Proliferation Reagent (WST-1) were from Roche Diagnostics (Mannheim, Germany).

### *Cell culture*

Hamster sebaceous gland cells were obtained from Kurabo Industries (Osaka, Japan). Sebaceous gland cells ($3.7 \times 10^4$ cells/cm$^2$) were cultured in phenol red-free DMEM/F12-Ham (1:1) supplemented with 10% FBS (v/v), 4 mM L-glutamine, 100 U/ml penicillin, 100 $\mu$g/ml streptomycin and 10 ng/ml EGF and maintained at 37°C in a humidified atmosphere containing 5% (v/v) $CO_2$. Three days after achieving confluence, sebaceous gland cells were differentiated by the treatment with 10 $\mu$g/ml insulin instead of EGF. The medium was then replaced every 2 days.

The cells were treated with various saccharides in the presence of insulin-containing differentiation medium for 8–14 days. In some assays, the cells were pretreated with TAK-242 or TPCA-1 1 hour prior to the addition of saccharides. These inhibitors were dissolved in DMSO and the final concentration of DMSO in the medium was <0.1%.

### *Oil red O staining*

Sebaceous gland cells were washed twice with PBS and fixed with 10% (v/v) formaldehyde in PBS for 15 min at room temperature. The cells were washed twice with distilled water and treated with 60% isopropanol (v/v) for 1 min, followed by the staining with 0.18% (w/v) oil red O in 60% isopropanol for 30 min at room temperature. The stained cells were washed twice with 60% isopropanol, treated with distilled water and observed with an IX73 light microscope (Olympus Optical, Tokyo, Japan) equipped with a DP73 digital camera (Olympus Optical, Tokyo, Japan). Oil red O was then extracted from the cells with 100% isopropanol and quantified by measuring the absorbance at 530 nm using an EONC microplate reader (Bio Tek Instruments, Winooski, VT, USA).

### *Quantitative RT-PCR*

For quantitative real time (qRT)-PCR, sebaceous gland cells were cultured in the differentiation medium for 8 days.



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**Table 1. Primers used for qRT-PCR**

| Gene (hamster) | Direction | Sequence | Accession number |
|---|---|---|---|
| TLR4 | Forward | 5′-CTCAGAGAGAGCCAGTGGAA-3′ | (*39*) |
| | Reverse | 5′-GGAGCATTAGTGAACCCTCG-3′ | |
| PPARγ | Forward | 5′-TCTCCTGTTGACCCAGAGCA-3′ | XM_040737946 |
| | Reverse | 5′-AAAGTTGGTGGGCCAGAATG-3′ | |
| SREBP1c | Forward | 5′-GCGGACGCAGTCTGGG-3′ | (*40*) |
| | Reverse | 5′-TGCCAATGTGTTTTCCCTGA-3′ | |
| ACC | Forward | 5′-ACACTGGCTGGCTGGACAG-3′ | (*40*) |
| | Reverse | 5′-CACACAACTCCCAACATGGTG-3′ | |
| FAS | Forward | 5′-AGCCCCTCAAGTGCACAGTG-3′ | (*40*) |
| | Reverse | 5′-TGCCAATGTGTTTTCCCTGA-3′ | |
| G6PD | Forward | 5′-AGAGAGGCCCCAGCCTATTC-3′ | XM_005086899 |
| | Reverse | 5′-TGTGAGGGTTCACCCACTTG-3′ | |
| GLUT4 | Forward | 5′-TATTTGCCGTCCTCCTGCTTG-3′ | XM_005067520 |
| | Reverse | 5′-TGGGTTTCACCTCCTGCTCTA-3′ | |
| GLUT1 | Forward | 5′-GCTGCCCTGGATGTCCTATC-3′ | XM_005083859 |
| | Reverse | 5′-CCACAATGAACCATGGGATG-3′ | |
| PK | Forward | 5′-TGGAATGAATGTGGCTCGTC-3′ | XM_040729809 |
| | Reverse | 5′-GGTCCCTTTGTGTCCAGAGC-3′ | |
| β-Actin | Forward | 5′-ACGAGGCCCAGAGCAAGAG-3′ | (*41*) |
| | Reverse | 5′-GGTGTGGTGCCAGATCTTCTC-3′ | |

On the ninth day, total RNA was isolated using NucleoSpin RNA and cDNA was generated using PrimeScript RT Master Mix. qRT-PCR was performed using TB Green Premix Ex Taq II in a Thermal Cycler Dice Real Time Systems II (Takara Bio Inc., Kusatsu, Japan). All reactions were performed in triplicate and analysed using the ΔΔCT method (*38*). The results were expressed as relative values normalized to β-actin. The primer sequences used are shown in Table 1.

#### *Measurement of glucose and lactate levels and LDH activity*

The culture supernatants of the cells differentiated by insulin were collected every time the medium is changed. The concentrations of glucose and lactate in the supernatants were then quantified using an Experimental Animal Glucometer (ForaCare Inc., Moorpark, CA, USA) and Lactate Assay Kit-WST, respectively. The intracellular LDH activity of the sebaceous gland cells on the ninth day was measured with Cytotoxicity Detection Kit[PLUS] (LDH).

#### *Measurement of cell proliferation*

Sebaceous gland cells were incubated with 10% WST-1/medium for 20 min at 37°C in a humidified atmosphere containing 5% (v/v) $CO_2$. The medium samples were then placed in 96-well plates and the absorbance at 450 nm and 650 nm (a reference) were measured using a microplate reader.

#### *Statistical analysis*

Data were analysed statistically using StatMateV (ATMS, Chiba, Japan). All groups were compared using the Tukey method. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

## Results

#### *Effects of polysaccharides on lipid synthesis in sebaceous gland cells*

The sebaceous gland cells from hamster auricles were differentiated to lipid droplet-forming cells by the treatment of insulin according to the manufacturer's instruction. During the differentiation period, five types of polysaccharides (0.1 or 1 mg/ml) were added to the culture medium. When evaluated by oil red O staining, the intracellular lipid levels were found to be significantly suppressed by the addition of 0.1 or 1 mg/ml of β-1,4-galactan (lupin, average molecular weight 1182 kDa) or 1 mg/ml of type II arabinogalactan, whereas rhamnogalacturonan I, arabinoxylan and β-glucan had little effects at either concentration (Fig. 1A, B).

Further examination in a wide range of concentrations confirmed that β-1,4-galactan and type II arabinogalactan dose-dependently suppress the lipid synthesis up to 20–30% of the control (Fig. 1C, D). β-1,4-Galactan was more potent than type II arabinogalactan, and the significant inhibitory effect of the former polysaccharide was seen at 0.1 μg/ml and higher concentrations. In contrast, 1 mg/ml of L-arabinose and D-galactose, both of which are main monosaccharides composing these two polysaccharides, did not show such inhibitory effects (Fig. 1E).

#### *Involvement of TLR4 signalling pathway in the effect of β-1,4-galactan*

For further analysis, we focused on β-1,4-galactan. Previously, β-1,4-galactan was reported to show immunostimulatory activity via TLR4 in macrophages (*42*), and type II arabinogalactan was to stimulate the maturation of dendritic cells via the same receptor (*43*). Furthermore, TLR4 was shown to be expressed in human sebaceous gland cells (*44*). Therefore, we evaluated whether TLR4 is involved in the effect of β-1,4-galactan in hamster sebaceous gland cells.

We first confirmed the expression of TLR4 in the cells by qRT-PCR (Fig. 2A). Interestingly, the mRNA level was increased nearly three times in the presence of LPS (50 ng/ml), a well-known ligand for TLR4, and β-1,4-galactan (10 μg/ml). We next treated the cells with different concentrations of LPS. LPS potently suppressed lipid synthesis at as low as 5 ng/ml (Fig. 2B). Moreover, TAK-242, a TLR4 signalling inhibitor (*45*), counteracted this



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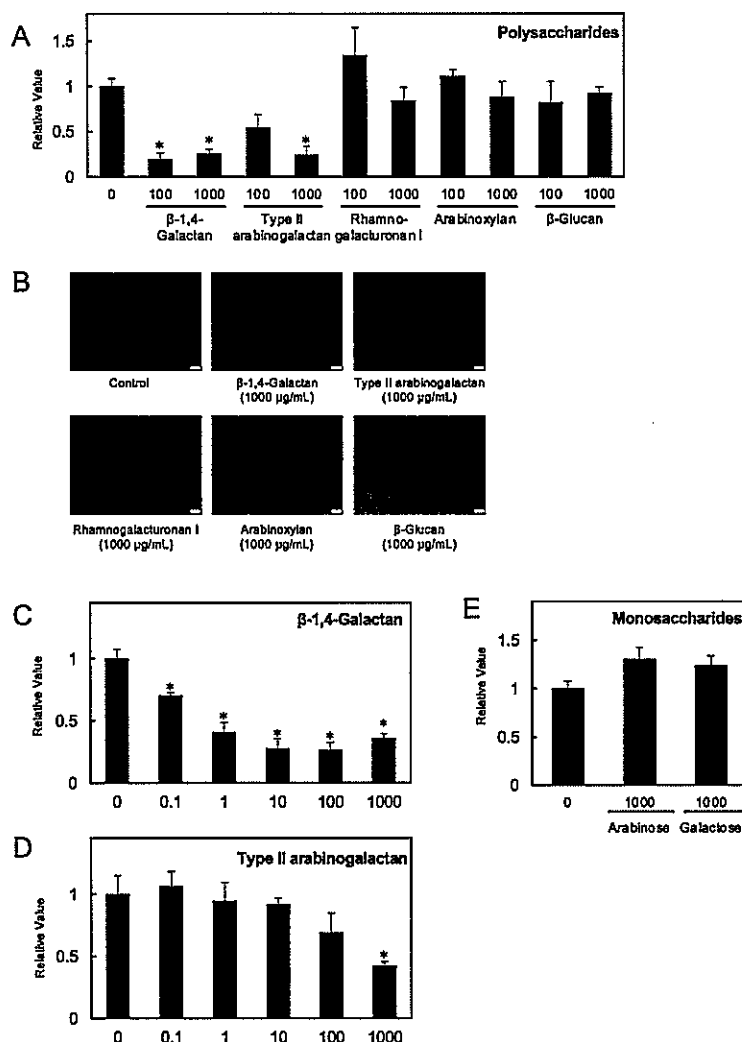


**Fig. 1. Effects of polysaccharides on lipid synthesis in sebaceous gland cells.** Sebaceous gland cells were cultured in the presence of the indicated polysaccharides (A–D) or monosaccharides (E), followed by the staining with oil red O. In (A) and (C–E), oil red O was extracted from the cells and spectrometrically quantified. The concentrations ($\mu$g/ml) of saccharides used are indicated in each panel. All experiments were performed at least three times and relative values are shown (mean values ± S.D.) *$P < 0.01$ between each saccharide-treated group and control (Tukey test). Bar = 100 $\mu$m (B).

suppressive effect in a concentration-dependent manner (Fig. 2C). Importantly, TAK-242 also counteracted the suppressive effect of $\beta$-1,4-galactan (Fig. 2C). In addition, TPCA-1, a potent and selective inhibitor of I$\kappa$B kinase $\beta$ (IKK$\beta$) (*46, 47*), counteracted the effects of LPS and $\beta$-1,4-galactan in a concentration-dependent manner (Fig. 2D). Since IKK$\beta$ is a downstream factor in the TLR4 signalling pathway (*48*), these results suggested that LPS suppresses lipid synthesis in sebaceous gland cells via the TLR4/IKK$\beta$ signalling pathway and that the effect of $\beta$-1,4-galactan is also mediated by the same signalling pathway.

#### *Regulation of lipid metabolism-related genes by $\beta$-1,4-galactan*

To further investigate the effects of $\beta$-1,4-galactan and LPS on lipid biosynthesis, we analysed the expression levels in sebaceous gland cells of the transcription factors and enzymes involved in lipid biosynthesis by qRT-PCR. $\beta$-1,4-Galactan (10 $\mu$g/ml) and LPS (50 ng/ml) significantly decreased the mRNA levels of PPAR$\gamma$, sterol regulatory element-binding protein 1c (SREBP1c), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (Fig. 3). These results suggested that these polysaccharides down-regulate the lipid biosynthesis at transcriptional levels.

#### *Effect of $\beta$-1,4-galactan on glucose metabolism*

We also examined the effect of $\beta$-1,4-galactan on glucose metabolism by analysing the expression levels of glucose transporters and enzymes (Fig. 3). $\beta$-1,4-Galactan and LPS significantly suppressed the expressions of glucose-6-phosphate dehydrogenase (G6PD), which supplies NADPH for lipid biosynthesis, and GLUT4, an insulin-dependent glucose transporter. In contrast, the mRNA levels of GLUT1, a constitutively active glucose transporter, and pyruvate kinase (PK), a glycolytic enzyme, were significantly increased.



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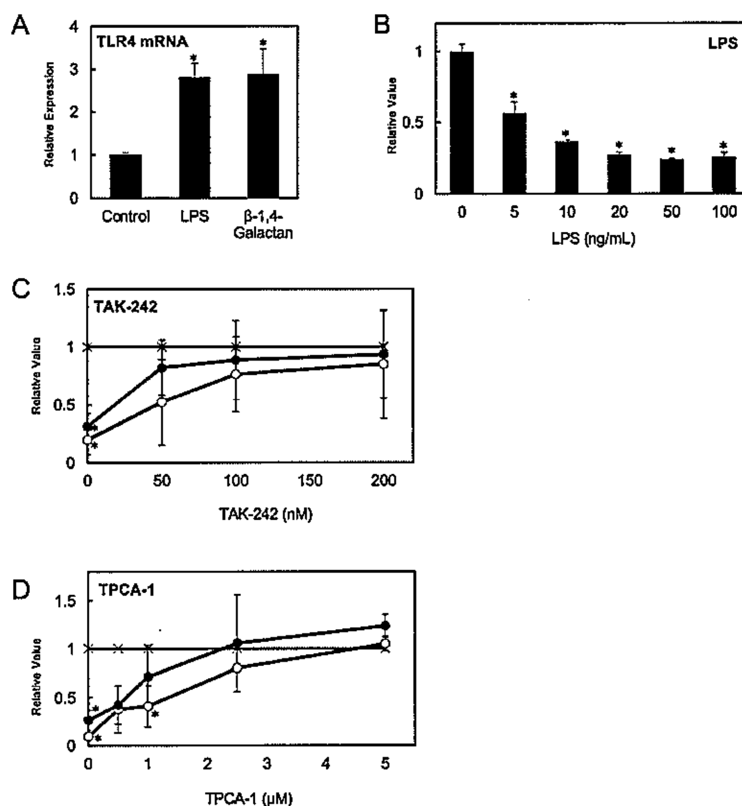


**Fig. 2. Involvement of TLR4 in the effect of β-1,4-galactan.** Sebaceous gland cells were cultured in the presence of β-1,4-galactan (10 μg/ml) or LPS (50 ng/ml) or in their absence, and the mRNA levels of TLR4 in the cells were analysed by qRT-PCR (A). The cells were cultured in the presence of different concentrations of LPS and the lipid contents were quantified by oil red O staining (B). Different concentrations of TAK-242 (C) or TPCA-1 (D) were added to the medium together with β-1,4-galactan (10 μg/ml) (closed circles), LPS (50 ng/ml) (open circles) or nothing (crosses), followed by oil red O staining. All assays were performed at least three times and relative values are shown (mean values ± S.D.) *$P < 0.05$ between each saccharide-treated group and control (Tukey test).

We further measured the glucose consumption of sebaceous gland cells. When the cells were cultured in the presence of β-1,4-galactan (10 μg/ml) or LPS (50 ng/ml), the glucose consumption increased by about 2.6-fold and 2.4-fold, respectively, as compared with the control (Fig. 4A). We also examined the effects on anaerobic glycolysis by measuring the intracellular activity of LDH (Fig. 4B) and the amount of lactate released into the medium (Fig. 4C). The results showed that the addition of β-1,4-galactan and LPS increased the intracellular LDH activity by 4.4-fold and 4.6-fold and the amount of the released lactate by 10-fold and 9.6-fold, respectively. However, the addition of TAK-242 suppressed the stimulatory effects, suggesting that β-1,4-galactan and LPS enhance anaerobic glycolysis via TLR4 (Fig. 4A–C).

We also examined the effect on the proliferation of sebaceous gland cells. As measured by WST-1 cell proliferation assay, β-1,4-galactan and LPS increased the proliferative activity about 2-fold (Fig. 4D). In the presence of TAK-242, however, the stimulatory effects were suppressed. These results suggested the involvement of TLR4 signalling.

## Discussion

Sebaceous glands secrete sebum and are thus considered as one of the tissues essential for maintaining skin homeostasis (*3, 4*). The secretion of sebum from sebaceous gland cells is regulated by various hormones, drugs, vitamins and environmental factors (*8–15*). In this study, we discovered that the plant-derived polysaccharide arabinogalactan down-regulates sebum secretion by suppressing lipid synthesis in the sebaceous glands. We also offer a new concept of the inhibitory mechanism of lipid synthesis in sebaceous gland cells.

So far, the effects of arabinogalactan on the skin have been restricted to those on epidermal keratinocytes and fibroblasts (*31–33*). By using two types of arabinogalactans, namely β-1,4-galactan (the main polysaccharide chain of type I arabinogalactan) and type II arabinogalactan, we showed that arabinogalactan inhibits lipid synthesis in hamster sebaceous gland cells. We also suggested that the effect of β-1,4-galactan is mediated via TLR4, which is a membrane receptor involved in the innate immune response. In agreement with this finding, LPS, a well-known ligand of TLR4, also inhibited lipid synthesis in sebaceous gland cells. TLR4 is known to recognize not only LPS but also a variety of structurally unrelated ligands, which include polysaccharides such as fungal-derived mannan and glucuronoxylomannan (*49*). Arabinogalactans are the polysaccharides characterized by a high galactose content (>80%) and the presence of galactan chains. Thus, arabinogalactans may be placed in the polysaccharides functioning as a TLR4 ligand with



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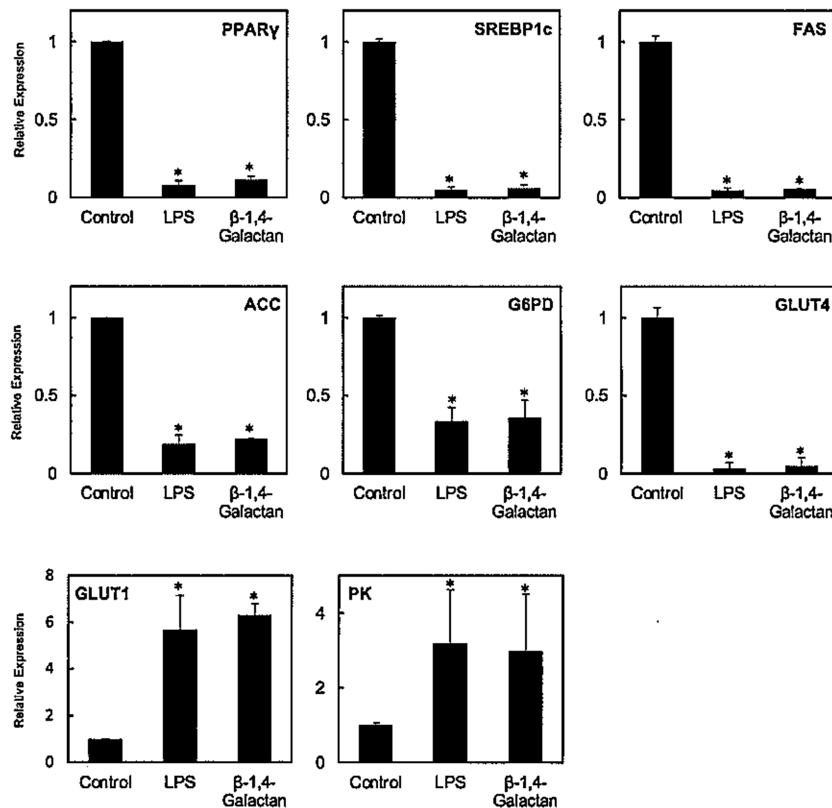


Fig. 3. Effect of β-1,4-galactan on the expression of the genes related to the metabolism of lipid and glucose. Sebaceous gland cells were cultured in the presence of β-1,4-galactan (10 μg/ml) or LPS (50 ng/ml), and the indicated mRNAs were quantified by qRT-PCR. All assays were performed at least three times and relative values are shown (mean values ± S.D.) $*P < 0.05$ between each saccharide-treated group and control (Tukey test).

much weaker agonist activity than LPS. Furthermore, to evaluate the functional importance of the backbone (poly-β-1,4-galactose structure), we enzymatically pretreated the β-1,4-galactan with arabinofuranosidase to remove the arabinose and arabinan side chains and tested the inhibitory effect of the side chain-free β-1,4-galactan on lipid synthesis. Since the enzyme-treated β-1,4-galactan still showed the inhibitory effect, the structure required for this effect was suggested to be the poly-β-1,4-galactose backbone (Ayaki et al., data not shown).

We showed that β-1,4-galactan and LPS up-regulate TLR4 mRNA in sebaceous gland cells (Fig. 2A). According to previous reports, LPS causes either the induction or suppression of the TLR4 mRNA expression, depending on cell types. For example, LPS induced the TLR4 mRNA expression in myocardial cells (*50*), vascular endothelial cells (*51*) and monocytes (*52*), while LPS suppressed it in mouse RAW264.7 macrophage cells (*53*). We think that in sebaceous cells the up-regulation of TLR4 mRNA by β-1,4-galactan and LPS strengthens the TLR4-mediated suppression of lipid biosynthesis. Since sebum may serve as energy source for microorganisms, the resultant decrease of sebum may be useful to suppress the growth of *Cutibacterium acnes* and *Malassezia* yeast, which cause acne vulgaris and seborrheic dermatitis, respectively (*5–7*).

In accordance with the suppression of lipid synthesis by β-1,4-galactan and LPS, two fatty acid-biosynthesizing enzymes, ACC and FAS were down-regulated by these two substances. In addition, G6PD, which supplies NADPH for fatty acid biosynthesis, was also down-regulated. PPARγ and SREBP1c, both of which are transcription factors to up-regulate ACC, FAS and G6PD (*54, 55*), were also down-regulated. These results suggested that β-1,4-galactan and LPS suppress lipid biosynthesis through the down-regulation of PPARγ and SREBP1c.

In our culture system, the sebaceous gland cells differentiate into lipid droplet-forming cells in the presence of insulin. The insulin signalling pathway is deeply involved in lipid synthesis (*56–58*). In this pathway, insulin activates phosphoinositide 3-kinase (PI3K) via the insulin receptor followed by the activation of Akt, and the activated PI3K and Akt subsequently enhance the expression of PPARγ and SREBP1c to promote lipid synthesis. (*56–58*). GLUT4, an insulin-dependent glucose transporter (*59*), was also down-regulated by β-1,4-galactan and LPS, suggesting that these polysaccharides have an inhibitory effect on the insulin signalling pathway.

Our results suggest the presence of crosstalk between the TLR4 signalling pathway and the insulin signalling pathway in the suppression of lipid synthesis by β-1,4-galactan (Fig. 5). From the results of Fig. 2D, IKKβ, a factor in the TLR4 signalling pathway, was suggested to be involved in the effect of β-1,4-galactan. It was recently reported that in adipocytes the phosphorylated IKKβ phosphorylates Ser-307 of insulin receptor substrate (IRS)-1, resulting in the interference with insulin-dependent



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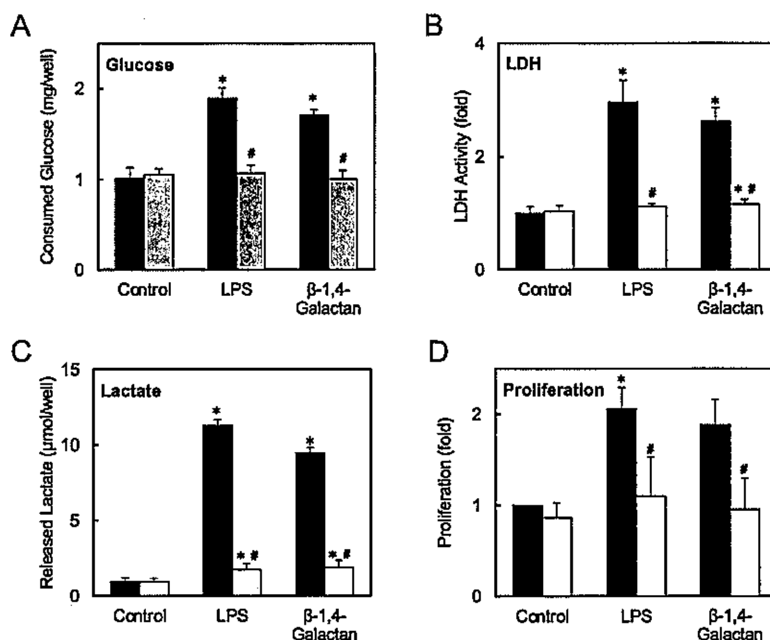


**Fig. 4. Effect of β-1,4-galactan on glycolysis.** Sebaceous gland cells were cultured in the presence of β-1,4-galactan (10 μg/ml) or LPS (50 ng/ml), followed by the measurements of glucose consumption (A), intracellular LDH activity (B), the amount of released lactate (C) and proliferation activity (D). The medium contained 100 nM TAK-242 (grey column) or did not (black column). All assays were performed at least three times and mean values ± S.D. are shown. *$P < 0.05$ between each saccharide-treated group and nontreated group in TAK-242-treated group or nontreated group (Tukey test). #$P < 0.01$ between TAK-242-treated group and nontreated group in each saccharide-treated group (Tukey test).

phosphorylation of the specific tyrosine residues of IRS-1 (*60, 61*). This interference was considered as a mechanism of insulin resistance. We thus speculate that in sebaceous gland cells, β-1,4-galactan first binds to TLR4 and then suppresses lipid synthesis through the phosphorylations of IKKβ and Ser-307 of IRS-1, resulting in the inhibition of the insulin signal transduction. However, further examinations are required to confirm this speculation.

It is well known that inflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumour necrosis factor-α are released when IKKβ and nuclear factor-kappa B (NFκB) are activated in the TLR4 signalling pathway (*63,64*). Therefore, it was likely that β-1,4-galactan and LPS release cytokines in sebaceous gland cells. When we treated hamster sebaceous gland cells with LPS (50 ng/ml) or β-1,4-galactan (10 μg/ml), the release of IL-1β and IL-6 were below the detection limit (data not shown). However, since LPS was previously reported to increase the expression levels of IL-6 and IL-8 in human sebaceous gland cells (*65*) and since a possible unexpected effect of the TLR4 signalling pathway was seen in autoimmune diseases (*66*), we should carefully consider the application of β-1,4-galactan to humans.

Apart from the suppression of lipid biosynthesis, β-1,4-galactan and LPS up-regulated anaerobic glycolysis in sebaceous gland cells as shown by the enhanced expression of PK and GLUT1 and the increase in glucose consumption, LDH activity and lactate release. Moreover, the suppressive effect of the TLR4 inhibitor TAK-242 suggested that the increase in glucose consumption, LDH activity and released lactate occur via the TLR4 signalling pathway. This effect seemed unique because it was earlier reported that levonorgestrel, a synthetic progesterone, concurrently suppressed glucose uptake and lipid synthesis in sebaceous gland cells (*67*). In addition, this action of LPS may be specific to sebaceous gland cells since the treatment of adipocytes with LPS suppressed both glucose uptake and lipid synthesis (*68*). However, it remained unclear whether this effect of β-1,4-galactan is seen only in sebaceous gland cells.

In addition, β-1,4-galactan and LPS stimulated the proliferation of sebaceous gland cells. This growth-stimulatory effect has not been reported with retinoic acid, another lipid synthesis inhibitor (*69,70*). Moreover, this effect was also suggested to occur through the TLR4 signalling pathway since the activity was suppressed by the TLR4 inhibitor TAK-242. In addition to IKKβ and NFκB, mitogen-activated protein kinases (MAPKs), including c-jun N-terminal kinase, extracellular signal-regulated kinase (ERK) and p38, exist downstream of the TLR4 pathway (*48*). Considering that ERK is well known as a kinase involved in promoting cell proliferation and survival (*71*), the activation of ERK may be involved in the stimulation of cell proliferation by β-1,4-galactan (Fig. 5). The up-regulation of anaerobic glycolysis should be useful for the cell proliferation.

Acne vulgaris is caused by increased sebum secretion, obstruction of skin pores and growth of *Cutibacterium acnes*, while seborrheic dermatitis is by increased sebum secretion and overgrowth of *Malassezia* yeast (*5–7*). Due to the lipogenesis-inhibiting activity in sebaceous gland cells, β-1,4-galactan may be useful for the treatments of these skin diseases. β-1,4-Galactan may also improve oily skin from a cosmetic point of view. In addition, the lactic acid



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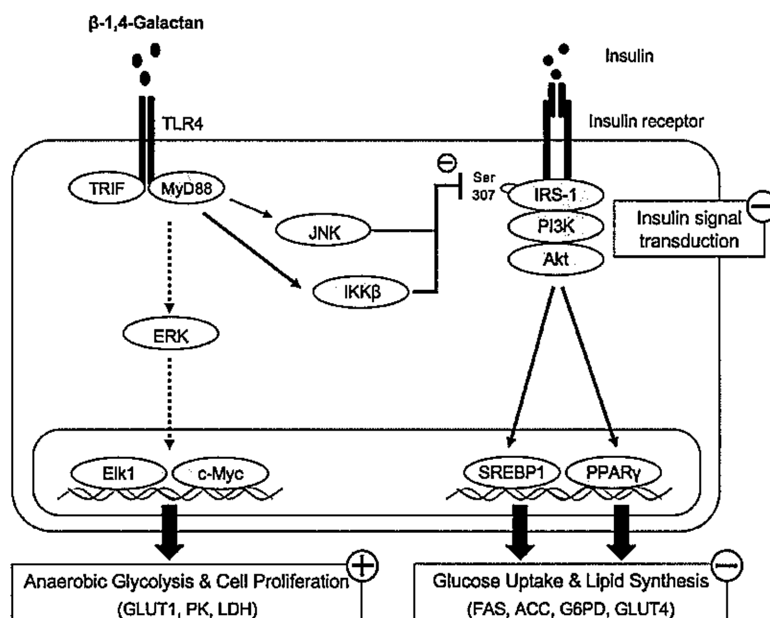


**Fig. 5. Possible signal pathways involved in the effect of β-1,4-galactan on lipid and glucose metabolism in sebaceous gland cells.** The signal pathway map was created based on the references (*48,57,60,62*). '+' and '-' indicate enhancement and suppression, respectively. TRIF, TIR-domain-containing adapter-inducing interferon-β; MyD88, myeloid differentiation factor 88.

extracellularly released from sebaceous gland cells may have some beneficial effects on skin as a natural moisturizing factor (*72*), a differentiation inducer for epidermal keratinocytes leading to the formation of skin barrier (*73*), a weak acid suppressing the growth of indigenous bacteria such as inflammatory *Staphylococcus aureus* that cause skin inflammation (*74,75*), and a weak peeling agent to keep the skin smooth (*76*). Previously, arabinogalactan has been reported to promote barrier formation by inducing the differentiation of epidermal keratinocytes (*32*) and to accelerate wound healing by proliferation of fibroblasts (*31*). Taken together, these results suggest that β-1,4-galactan has potential as a material with therapeutic and cosmetic values for the skin.

In conclusion, we showed for the first time that β-1,4-galactan suppresses lipid synthesis in sebaceous gland cells. The action was suggested to be mediated by TLR4 and to be exerted by inhibiting the insulin signal transduction. Furthermore, we revealed that β-1,4-galactan promotes the production of lactic acid in sebaceous gland cells. β-1,4-Galactan was thus expected to have beneficial effects on the skin.

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## Data Availability Statement

Data will be made available on request.

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## Conflict of Interest

Satomi Ayaki, Takahito Tokuyama and Takashi Tokuyama have a patent (JP_007102591).

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