이학석사 학위논문

Role of Fucosyltransferase 1 in inflammatory skin responses

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by

Kyeong Hyeon Kim

A thesis submitted to the Department of Biomedical Sciences in partial fulfillment of the requirements for the Degree of Master of Science at Seoul National University College of Medicine

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Approved by Thesis Committee:

Professor _____ Chairman

Professor _____ Vice chairman

Professor _____

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부위원장 정진호 (인)

위 원 조 정현 (인)

Abstract

Role of fucosyltransferase 1

in inflammatory skin responses

Kyeong Hyeon Kim

Department of Biomedical Sciences

Major in Biomedical Sciences

Seoul National University Graduate School

ABH antigens are representative terminal structures of glycan chain. Since the discovery of ABO blood groups on human erythrocytes by Karl Landsteiner, it has been found that ABH antigens are also present in biological fluids and various tissues including skin. ABH antigens are synthesized from

the addition of monosaccharide units to the H precursors in a step-wise manner. To be more specific, H antigen synthesis requires the addition of a fucose onto precursors and the catalysis of this reaction by α-1,2-fucosyltransferase 1 (FUT1). After having synthesized H antigen, A and B antigens can be made by adding corresponding sugars to H antigen. It has been elucidated that FUT1 is over-expressed or under-expressed in some cancers and has an important role in tumor growth and metastasis. Moreover, FUT1 has also been found to mediate angiogenesis and inflammatory cell adhesion. Ultraviolet light (UV) exposure on skin can cause acute skin inflammation as well as photodamage and skin tumors. The Oxazolone is the chemical that induces allergic contact dermatitis (ACD). The 12-O-tetradecanoylphorbol 13-acetate (TPA) is the chemical that induces skin inflammation which can represent a model of irritant contact dermatitis (ICD). The mechanisms of UV or chemicals such as oxazolone and TPA induced skin damage and inflammatory responses have been widely characterized. However, the role of FUT1 in these kinds of skin inflammation model has yet to be determined. First, I compared the UV-induced inflammatory responses between FUT1 KO and wild type mice. CD45-positive inflammatory cells were significantly more recruited in UV-irradiated skin of FUT1 KO mice. Because no significant difference between FUT1 KO mice and its wild type littermates was detected in the double immunofluorescent staining with CD31, a blood vessel marker, and CD45,

more infiltration of CD45-positive cells into the skin is independent of extravasation. UV-induced pro-inflammatory cytokine, IL-1β, was significantly augmented in FUT1 KO mice when compared to wild type mice. Anti-inflammatory cytokine, IL-10, did not show any differences between two genotypes. VEGF and ICAM-1, known to be previously associated with FUT1, were also evaluated. Although the level of VEGF did not show any differences between the two genotypes, the levels of UV-induced ICAM-1 were significantly increased in FUT1 KO mice, compared to wild type littermates. These results indicate that mice deficient in FUT1 are more susceptible to UV-induced inflammation. Next, by using oxazolone-induced allergic contact dermatitis model and TPA-induced irritant contact dermatitis model, I found that mice deficient in FUT1 KO showed different responses with wild type mice. Oxazolone-induced immune cell infiltration were more increased in FUT1 KO mice. TPA-induced ICAM-1 expression in the epidermis was highly decreased in FUT1 KO mice compared to that of the wild type littermates. Further studies using two types of contact dermatitis model should be conducted for exact results. In conclusion, FUT1 deficiency can have an effect on skin inflammation *in vivo*, suggesting the complex role of FUT1 in the context of distinct inflammatory conditions.

Key words: Ultraviolet radiation, TPA, inflammation, FUT1, cell adhesion molecules

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Introduction

1. Fucosyltransferase-1 (FUT1)

Cells are composed of 4 building blocks: proteins, nucleic acids, lipids, and glycans. Glycans, referred to as carbohydrates, consist of saccharides and are typically linked to lipids and proteins. Glycans are involved in various biological processes and physiological systems and regulate protein-protein and cell-cell interactions (Lowe & Marth, 2003). Enzymes named glycosyltransferases attach glycans to various organic molecules as well as lipids and proteins. This enzymatic procedure is called glycosylation. In the endoplasmic reticulum and the Golgi apparatus, N-linked and/or O-linked glycan chains are synthesized and attached to proteins (Dunphy et al, 1985). Glycosylation is one of the most common type of posttranslational modification (Hart, 1992).

Fucosylation, a type of glycosylation, is the enzymatic process of adding fucose sugar units to a molecule. This procedure can be done by enzymes called fucosyltransferases (FUTs) (Aplin & Jones, 2012). Based on their acceptor specificity and protein sequence, they are classified into two main families. The first group is α(1,2) fucosyltransferases (FUT1 and FUT2) involved in the synthesis of H antigen which are required to synthesize A and B antigens. The other group is α-1,3-fucosyltransferases (FUT3, FUT4, FUT5,

FUT6, and FUT7) required in the synthesis of Lewis-related antigens (Mas et al, 1998).

ABH antigens are representative terminal structures of glycan chains (Hakomori, 1999). Since the discovery of ABO blood groups on human erythrocytes by Karl Landsteiner (Yamamoto, 2004), it has been elucidated that the same molecules could be also present in tissues and biological fluids **(Table I).** This is the reason why the term, 'histo-blood group antigens', is widely used for emphasizing their roles in tissues (Marionneau et al, 2001; Ravn & Dabelsteen, 2000). Several studies have shown that the antigen changes seen in tumours often involve histo-blood group antigens, and that some of these antigens are correlated with prognosis (Ravn & Dabelsteen, 2000).

Table I. Tissue distribution of blood group antigens

Tissue	Carbohydrate expression
Skin(keratinized)	A/B antigen, H type 2
Gastric Mucosa	Lewis a/b, A/B, H, sialyl Lewis a(SLe a)
Small intestine	A/B, Lewis b/y/a/x
Large intestine	Lewis b/a/x/y, A, H type 2
Breast	A/B,H, Lewis b/a/x/y, sialyl Lewis a
Salivary Glands	H, Lewis y/b, A/B
Liver	Lewis y/a/b, A/B, H,
Kidney	Lewis x, sialyl Lewis a, A/B, H
Thymus	A/B, H, Lewis y/x

Biosynthesis of ABH antigens proceeds from the stepwise addition of monosaccharide units to the precursors. For synthesizing H antigen, addition of a fucose onto precursors should be catalyzed by α-1,2-fucosyltransferases. This enzyme transfers a fucose from GDP-fucose onto the galactose of terminal disaccharide, which are classified into type I (Gal-β1,3-GlcNAc) and type II (Gal-β1,4-GlcNAc) (Mas et al, 1998). After synthesizing H antigen, A and B antigens can be made by adding sugars to H antigens. The difference between A and B antigens is determined by the terminal sugars, N-acetyl-D-galactosamine and D-galatose respectively (Yamamoto, 2004). In humans, as mentioned, two types of α-1,2-fucosyltransferases are known to participate in the synthesis of H antigens, FUT1 and FUT2. Each enzyme is encoded by two distinct genes, *fut1 (H gene)* and *fut2(secretor gene, se)*. The absence of each gene leads to different results. Lack of a functional *fut1* allele is mainly characterized by the rare Bombay phenotype in which erythrocytes does not express ABH antigens on their membranes. On the other hand, the lack of functional *fut2* allele causes a non-secretor phenotype characterized by the absence of ABH antigens in saliva and on various epithelial cell types (Marionneau et al, 2001). Unlike the Bombay phenotype, non-secretor phenotype is not unusual. Around 20% of Europeans and North Americans possess non-secretor phenotype (Oriol et al, 2000).

The α-1,3/4-fucosyltransferases group (FUT3, FUT4, FUT5, FUT6, and FUT7) contributes to the synthesis of Lewis x, Lewis y, Lewis a, Lewis b, sialyl-Lewis x, and sialyl-Lewis a **(Figure 1).** These fucosylated oligosaccharide structures are considered as tumor-associated antigens. This is because they are involved in tumor cell invasion and metastasis. When tumor cells are attached to endothelial cells, two adhesion molecules, E-selectin and P-selectin, expressed on the plasma membrane of activated endothelial cells are involved. In their amino-terminal, these adhesion molecules have calcium ion-dependent lectin domain that recognizes sialylated and fucosylated oligosaccharide determinants expressed preferentially in human cancer cell line such as pancreatic (BxPC3), hepatic (HepG2), and colonic (HT-29) (Mathieu et al, 2004).

The expression changes of blood group antigens in carcinomas are tissue-specific (Grekov et al, 1978; Ichihara et al, 1993; Terada et al, 1991)(Table II). For example, in gastric cancers, loss of blood group A,B,H and Lewis b antigens are detected. However, in colonic cancer, these antigens are highly increased (Greenwell, 1997). There are no generalized patterns of changes in antigen expressions related with cancers.

Figure 1. The synthesis of ABH antigens

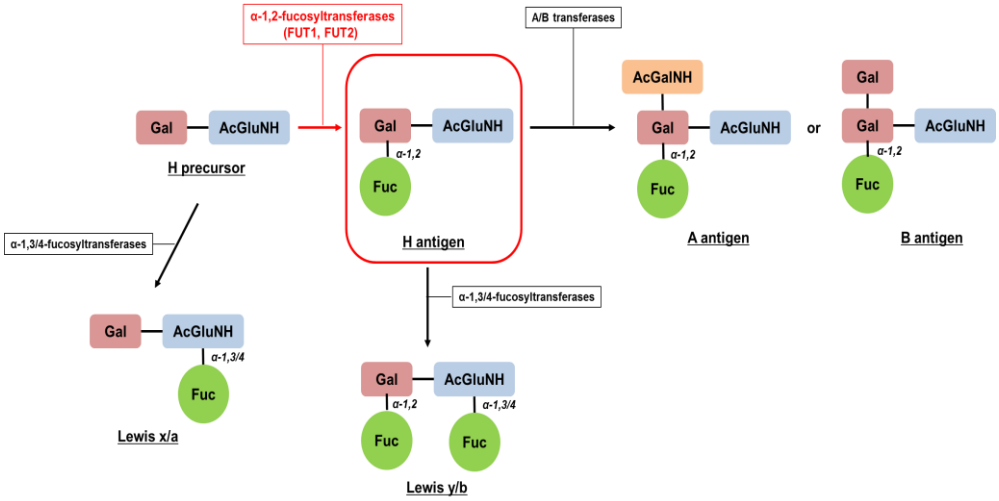

Table II. Tissue-specific expression changes in blood group expression

Carcinoma tissues/cells	Changes in ABH antigens
Colon	↑ ABH (re-expression)
Gastric	↓ ABH (loss), ↑ Lewis a (re-expression)
Bladder	↓ ABH (loss), Lewis a (appearance)
Lung	↓ ABH (loss)
Breast	↓ H and Lewis b (loss)
Liver	↑ ABH (increased)
Erythrocytes	↓ ABH (decreased) ; Erythrocytes from leukemia
Endometrium	↑ ABH (appearance)
Epithelium	↑ABH (increased) ; Cutaneous epithelioid

2. Ultraviolet(UV)-induced skin inflammation

2.1. Ultraviolet radiation (UVR) and skin damage

UVR is comprised of UVA (320–400 nm), UVB (290–320 nm) and UVC (200–290 nm). UVC, the most mutagenic radiation, hardly reach the earth's surface as almost all of UVC is absorbed by the ozone layer (Diffey, 2002). However, a significant proportion of the UVA and UVB spectrum reach the earth and penetrate the epidermal layers. Although UVB makes up a minor portion, below 5% of the sun's irradiation on earth, it is well characterized as a mutagenic component (Agar et al, 2004).

UVR photodamage can occur either directly or indirectly. If the energy of UV is directly absorbed, it causes biochemical reactions, resulting in molecular changes. Not only that, but this energy can produce reactive oxygen species (ROS), which acts as mediators of indirect photodamage. ROS accumulation leads to oxidative stress and adduct formation of biomolecules. DNA has the ability to absorb a wide spectrum of UV wavelengths. Therefore, DNA is a direct target of UV damage, forming photoproducts such as cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs) by UVB and UVA, respectively. These photoproducts interfere with biological processes and induce various signals resulting in cell survival or cell death. Cell survival is through the repair processes induced by photoproducts. Cell

death is caused by photodamage-induced apoptotic signaling pathway (Jans et al, 2006).

2.2. UVR-induced inflammasome activation

Once epidermal keratinocytes get damaged by UVR, one of the earliest responses is the inflammasome formation within their cytoplasm (Feldmeyer et al, 2007). Inflammasome is a multi-protein molecular scaffold that is responsible for caspase-1 activation (Dowling & O'Neill, 2012). A highly conserved intracellular receptor family termed nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs) is the main component of the inflammasome. Among the several NLR family members, it is well known that UV radiation activates NLRP3. NLRs can recognize damage associated with molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs), depending on level of specificity.

The inflammasome complex induced by UVR recruits inactive procaspase-1 molecules and processes them into active caspase-1 molecules, which are required for the activation of the IL-1 family. For IL-1β and IL-18 to be in their mature form, caspase-1–mediated cleavage is the limiting step (Nasti & Timares, 2012). On the other hand, other IL-1 families such as IL-1α and IL-33, do not require direct caspase-1 processing for their activation. They

depend on inflammasome-associated unconventional mechanisms instead. In fact, IL-33 is negatively affected by caspase-1 cleavage and inactivated (Cayrol & Girard, 2009).

Pro-inflammatory cytokine IL-1β is tightly controlled by several endogenous inhibitors such as IL-1 receptor antagonist (IL-1Ra) and by processes like inflammasome and caspase-1 activation. The transcription of pro-IL-1β is controlled by nuclear factor–κB (NF-κB) and mitogen activated protein kinase (MAPK) signaling (Dowling & O'Neill, 2012).

The formation of inflammasome and the subsequent activation of IL-1 family regulate keratinocyte cell fate in terms of pathway determining the survival or the death of the cell. Moreover, IL-1 family members have been revealed to possess the ability to promote alternative pathways for T cell development.

3. Allergic contact dermatitis (ACD) model

Different types of inflammatory animal models can g ive a better understanding of the inflammatory process. They allow researchers to test their hypothesis and confirm the effect of a test compound against inflammatory conditions.

Allergic contact dermatitis (ACD) is classified as a delayed type hypersensitivity response. Animal model of this skin disease is called contact hypersensitivity (CHS) (Honda et al, 2013). Two phases named sensitization and challenge are required for CHS development.

In sensitiztion phase, chemicals called haptens can be used to induce CHS. The molecular mass of haptens are usually below 500. After haptens application, keratinocytes start to produce a various kinds of chemical mediators including TNF-α, IL-1β and prostaglandin E2. These mediators help skin DC maturation. Although the stratum corneum as well as tight junction in skin have a role as a skin barrier, haptens are small enough to penetrate across the skin. Sensitization of dermal DCs, innate immune system activation and mast cell activation are occurred (Honda et al, 2013).

In challenge phase, haptens should be applied to skin again. Haptens can activate both antigen-nonspecific and antigen-specific inflammation. Upon applying haptens, kertinocyte start to produce various proinflammatory cytokines, activating vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin. These kind of adhesion molecules attract T cells to skin tissues. Antigen-specific inflammation follows non-spicific inflammation. T cells which are infiltrated into skin are activated by cutaneous antigen-presenting cells. Cytokines

released by activated T cells can result in various inflammatory responses (Honda et al, 2013).

4. Irritant contact dermatitis (ICD) model

A 12-*O*-tetradecanoylphorbol 13-acetate (TPA), a tumor promoting agent and protein kinase C activator, is one of the chemicals that cause cutaneous inflammation when applied. Topical application of TPA was reported to induce a relatively longer-lasting inflammatory response than arachidonic acid (AA) (Rao et al, 1993).

Irritant contact dermatitis is a non-allergic local inflammatory response of the skin characterized by edema and erythema. An acute or chronic exposure of the chemical or physical irritant to the skin has been accounted to cause irritant contact dermatitis (Saary et al, 2005). TPA and diverse irritants such as 2,4-dinitrochlorobenzene (DNCB), 2,4-dinitrofluorobenzene (DNFB), and croton oil are able to induce irritant contact dermatitis in animal models (Veronesi et al, 1995). Damage or activation of epidermal cells caused by skin irritants results in irritant contact dermatitis. After the exposure of irritants on the skin, epidermal cells release proinflammatory cytokines and chemokines such as TNF-α, CXCL1 and MIP-2. These mediators lead to vasodilation and leukocyte infiltration, resulting in epidermal thickening, edema and erythema.

In this series of process, TNF-α has been shown to play an active role. TNF-α is a proinflammatory cytokine involved in a number of inflammatory diseases. TNF-α induces the expression of various adhesion molecules as well as chemokines. Adhesion molecules induced by TNF-α includes VCAM-1, ICAM-1 and E-selectin (Kupper, 1990). Adhesion molecules and chemokines recruit leukocytes such as T lymphocytes, neutrophils and infiltrated leukocytes which mediate inflammatory responses (Han et al, 2007).

Purposes

ABH antigens have been found in various tissues including skin, and biological fluids as well as on human erythrocytes (Ravn & Dabelsteen, 2000). The enzyme, FUT1, is essential for the synthesis of ABH antigens. It has been elucidated that FUT1 is over- or under-expressed in some cancers and has an important role in tumor growth and metastasis. Moreover, FUT1 mediates angiogenesis and inflammatory cell adhesion in the inflammatory diseases such as rheumatoid arthritis. Our group has previously found that FUT1 deficiency can decrease the expression of cell adhesion molecules which are induced by cytokines stimulation *in vitro*. This study has been performed to suggest *in vivo* evidence that FUT1 might play a role in inflammatory responses. To examine the role of FUT1 in various aspects of inflammation, different inflammatory stimuli such as UV irradiation or chemicals were used. Two types of chemicals, oxazolone and TPA were used for Allergic Contact Dermatitis model and Irritant Contact Dermatitis model.

Part I. UV-induced inflammatory responses in FUT1 KO mice

Part II. Oxazolone-induced inflammatory responses in FUT1 KO mice

Part III. TPA-induced inflammatory responses in FUT1 KO mice

Materials and Methods

Mice

FUT1 knock-out mice (C57BL/6J background) (Domino et al, 2001) were purchased from Jackson laboratory (Bar Harbor). Wild type counterpart mice used for comparison are littermates of FUT1 KO mice. Animals were bred in an AAALAC- accredited facility in Seoul National University Hospital. While 6- to 7-week old female mice in telogen hair cycle were used to test for UV-induced inflammatory responses, 8- to 10-week old female mice were used in the TPA-induced irritant contact dermatitis model. All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University Hospital.

UV irradiation to mice

Two days before UV irradiation, the hair on the dorsal side of the mice was shaved with a trimmer. For depilation, a commercial chemical depilatory cream called Veet (Oxy-Reckitt Benckieser, Seoul, Korea) was used. For UV exposure, a device UV-800 (Waldmann Co., Villingen-Schwenningen, Germany) equipped with a F75/85W/UV21 fluorescent sun lamp with an emission spectrum between 275 nm and 380 nm (peak at 300 nm-315 nm) was

utilized. A Kodacel filter (TA401/407, Kodak Co., NY) was used to remove UVC (wavelengths below 290 nm). The radiation intensity was measured 30 cm away from the light source, using a UV meter (585100, Waldmann Co.)The irradiation intensity came out to be approximately 0.5-0.6 mW/cm^2. Mice were first anesthetized by intraperitoneal injection of Zoletil (Zolazepam and Tiletamine) and then exposed to a single dose of UV (200 mJ/cm^2) on the dorsal skin. Skin samples were obtained 6, 24, and 48 hours after the UV irradiation. Control group samples were acquired from the non-irradiated mice. The dorsal skin fold thickness of each sample was measured using a digital caliper (Mitutoyo Co., Kanagawa, Japan).

Induction Contact hypersensitivity to mice

5 days before challenge, sensitization was conducted. Mice were anesthetized with isoflurane inhalation and 2% oxazolone (Sigma-Aldrich, MO) dissolved in vehicle (acetone and olive oil) was applied to abdomen (50 μl) and paw (5 μl).

On the day of challenge, 1% oxazolone was applied to right ear (10 μl to each surface). The same amount of vehicle was applied to the left ear for comparison. Mice were sacrificed 18 and 48 hours after challenge and ear biopsies were obtained and weighed. Whole ear was detached and cut off with an 8 mm punch (Kai Industries Co. Ltd., Gifu, Japan). At each indicated time point (18, 24, 42, 48 hours after challenge), the ear thickness was measured with a digital caliper.

Topical application of TPA to mice

Mice were anesthetized with isoflurane inhalation and 0.01% TPA (Sigma-Aldrich, MO) dissolved in 20 μl of acetone was applied to the inner and outer surfaces of the right ear (10 μl to each surface). DMSO dissolved in 20 μl of acetone was applied to the left ear for comparison. Mice were sacrificed 24 and 48 hours after TPA application and ear biopsies were obtained and weighed. Prior to TPA application and at each indicated time point (2, 6, 12, 24, 48 hours after TPA treatment), the ear thickness was measured with a digital caliper.

Quantitative real-time RT-PCR

Total RNA was isolated from tissue using RNAiso Plus (Takara Bio Inc., Shiga, Japan). The 1 μg of total RNA was converted to complementary DNA (cDNA) using a First Strand cDNA Synthesis Kit (MBI Fermentas, Lithuania). Quantitative analysis of mIL-1β, mIL-10, IL-1Ra, mNLRP3, mCXCL-1, mMCP-1, mVEGF, mICAM-1 and endogenous reference m36B4 cDNA was performed using the SYBR Premix Ex Taq II kit (Takara Bio Inc.) with a 7500 Real time PCR System (Applied Biosystems, CA). Sequence specific primer information is shown in **Table III.** PCR conditions were 50℃ for 2 min, 95℃ for 2 min, followed by 40 cycles at 95℃ for 15 s and 60℃ for 1 min. Data was analyzed using the $2^{-\Delta\Delta CT}$ methods and normalized to levels of m36B4 transcript.

ELISA

The protein quantity of IL-1β in mice was measured using mouse Fluorokine MultiAnalyteProfiling (MAP) bead-based assays (R&D systems, MN). The fluorescence intensity was read with Bioplex200 (Bio-rad, CA) according to the manufacturers' protocols.

Histological analysis of the skin

Obtained tissue samples were fixed in 10% neutralized formalin for 48h and embedded in a paraffin block. Tissue samples such as the dorsal skin and the ear skin were cut into 4 μm thick sections and mounted on silane-coated slides (Dako, Denmark). Tissue slides were subjected to hematoxylin and eosin (H&E) staining to analyze the thickness of the epidermis and the dermis and to compare the levels of inflammatory responses between various samples. Immunohistochemistry (IHC) and immunofluorescence (IF) staining with specific antibodies were also performed.

More specifically, for IHC staining of CD45 and ICAM-1, 4 μm thick sections were left in a 58℃ dry oven for 1 hour for deparaffination and were rehydrated using graded alcohols. For the antigen retrieval, sections were boiled in pH 6.0 TRS solution (S2031, Dako) for 10 min. Sections were then incubated with blocking solution for 30 minutes and stained with the monoclonal CD45 (1:100, 550539, BD Pharmingen, CA, USA) or ICAM-1(1:100, 550287, BD Pharmingen) antibody. After overnight incubation at 4 ℃, sections were rinsed with PBS and visualized with biotinylated secondary and horseradish-streptavidin conjugate served in a LSAB kit (Golden Bridge International Inc., WA). The chromogenic substrate was 3-

amino-9-ethylcarbazole. Sections were momentarily immersed in Mayer's hematoxylin for counterstaining.

For the IF staining, sections were stained with the monoclonal CD45 and CD31 (M3382, Spring Bioscience, CA) antibody overnight at 4 ℃. Afterwards, tissues were covered with Alexa-594 or 488 secondary antibody for 1 hour at room temperature. Before analysis under microscope, sections were sealed with aqueous mounting solution (S3025, Dako). H&E and IHC staining images were obtained with a 12.5 megapixel digital camera (DP70, NA=0.5, Olympus Optical Co., Japan) equipped with a light microscope (BX51, Olympus Optical Co.). IF staining images were taken by digital camera (DFC500, Olympus Optical Co.) with a fluorescence microscope (BX61, Olympus Optical Co.) using Leica Application Suite Advanced Fluorescence Software (LAS AF, Leica Microsystems, Switzerland). Epidermal thickness was evaluated by measuring the length of the epidermis between the basal layer and the base of the stratum corneum. Dermal thickness was determined by measuring the distance from the basal layer to the subcutaneous fat. The skin thickness and the number of CD45 stained cells were measured with Image J software (by Wayne Rasband, NIH).

Statistical analysis

For statistical significance testing, two-tailed Student's t-test was used. Significance was accepted when the p value was less than 0.05.

Table III. Primer sequences used in quantitative real-time RT-PCR

Gene	Foward	Reverse
m36b4	TGGGCTCCAAGCAGATGC	GGCTTCGCTGGCTCCCAC
mCXCL-1	CCGCAGGTCCAATTCACACT	GCGGCAGAGCAAAAG
mICAM-1	CCGCAGGTCCAATTCACACT	CAGAGCGGCAGAGCAAAAG
mIL-10	GGTTGCCAAGCCTTATCGGA	ACCTGCTCCACTGCCTTGCT
mIL-1Ra	AAATCTGCTGGGGACCT	TCTTCTAGTTTGATATTT
mIL-1β	GACTCATGGGATGATGATGATAAC	CCATACTTTAGGAAGACACGGATT
mMCP-1	CATCCACGTGTTGGCTCA	GATCATCTTGCTGGTGAATGAGT
mNLRP3	ATTACCCGCCCGAGAAAGG	TCGCAGCAAAGATCCACA
mVEGF	AGCGTTCACTGTGAGCCT	ACGCGAGTCTGTGTTTTTGC

Results

Part I. UV-induced inflammatory responses in FUT1 KO mice

I-1. H type 2 antigen synthetized by FUT1 (α-1,2-fucosyltransferase 1), was not detected in the skin of FUT1 KO mice

ABH antigens are known to present in the normal epidermis and in the epidermal appendages in humans. These are detected in the stratum corneum, stratum granulosum, stratum spinosum, acrosyringium, keratogenous zone of hair follicle, eccrine duct and eccrine gland (England et al, 1979).

The immunoreactivities to the H type 2 antigen were observed in the epidermis, hair follicles, sebaceous glands and blood vessels of wild type mice. However, these were not detected in the tissues of FUT1 KO mice **(Figure 2)**.

I-2. The skin fold thickness following UV irradiation was significantly increased, but the difference between wild type mice and FUT1 KO mice was negligible

The skin fold thickness was measured by a digital caliper at an indicated time point. Although both wild type and FUT1 KO mice in UV-irradiated group showed a significant increase in skin thicknesses **(Figure 3(a))**, there was no significant difference between wild type and FUT1 KO mice.

Epidermal and dermal thicknesses in H&E staining were histologically measured **(Figure 3(b), 3(c), 3(d))**. The thickness of the skin was increased until 48 hours after UV irradiation. Epidermal thickness in both wild type and FUT1 KO mice was increased about two times 48 hours post-irradiation.

Figure 2.

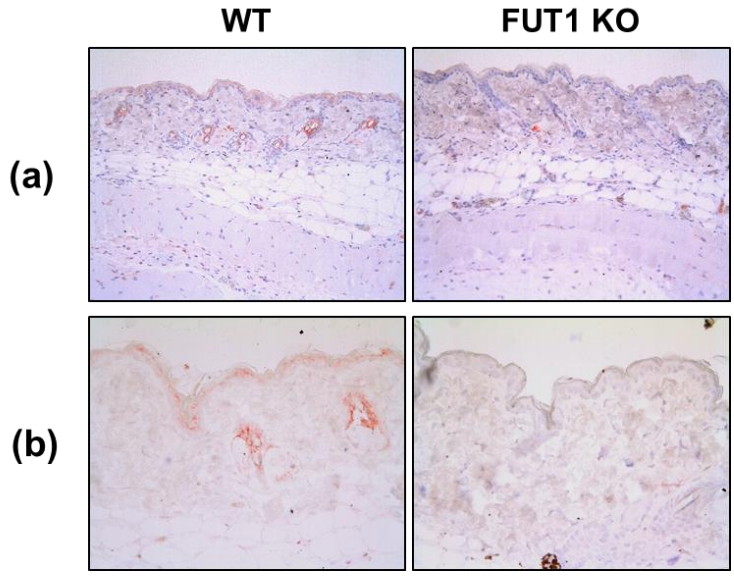

Figure 2. H type 2 antigen of ABO histo-blood group, synthetized by FUT1 (α-1,2-fucosyltransferase 1), was not detected in the skin of FUT1 KO mice.

H type 2 antigen of ABH blood group can be synthesized by FUT1. To check the expression of H type 2 in wild type (WT) and FUT1 KO mice, immunohistochemical staining was performed in non-irradiated skin. WT mice showed the immunoreactivities to the H type 2 antigen in the epidermis, hair follicles, sebaceous glands as well as blood vessels. On the other hands, these antigens were not detected in FUT1 KO mice. Original magnification x 200 **(a)** and x 400 **(b)**

Figure 3.

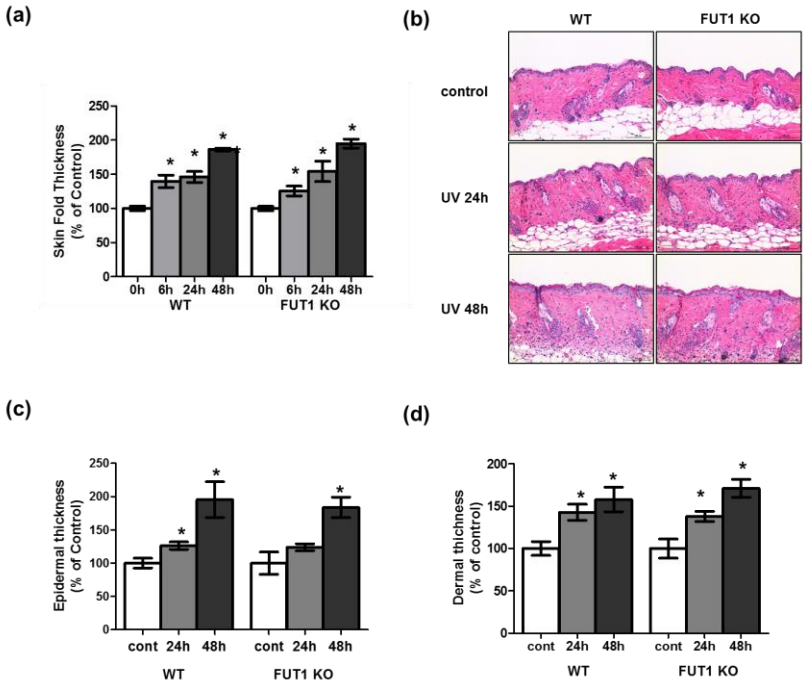

Figure 3. The skin fold thickness following UV irradiation was significantly increased, but the difference betweenwWT mice and FUT1 KO mice was negligible.

(a) The skin fold thickness of WT and FUT1 KO mice was measured by a digital caliper at 0h, 6hr, 24hr 48hr after UV irradiation (200mJ/cm^2). (b) Histological evaluation indicated that (c) epidermal and (d) dermal thickness were not significantly different between WT and FUT1 KO mice. (n=4~8 in each group. Error bars indicate standard error of the mean, *:$p<0.05$ vs. control (0h) by Student's T test)

I-3. Higher levels of inflammatory cell infiltration in UV-irradiated skin were observed in FUT1 KO mice, in comparison with WT mice

Leukocyte membranes contain more than 200 different proteins, most of them being glycosylated (Rudd et al, 1999). These glycosylation patterns are essential for the regulation of T-cell activation, migration, and apoptosis (Lowe, 2001). CD45 is one of the most abundant leukocyte transmembrane glycoproteins and is expressed on the surface of all hematopoietic lineage cells and their precursors, excluding mature erythrocytes and platelets. Therefore, CD45 has been used as pan-leukocyte marker. The number of CD45-positive cells infiltrated in skin tissues is related with how severe the inflammation is.

The expression levels of CD45 were examined in tissues by performing immunohistochemical staining **(Figure 4(a))**. Immunofluorescence staining of CD31 and CD45 was also performed to visualize CD45-positive cells adjacent to cutaneous blood vessels **(Figure 4(b))**. It was observed that CD45-positive cells were more infiltrated in the skin of FUT1 KO mice than that of WT mice at 48 hours post-irradiation **(Figure 4(c))**. These results show that UV-induced inflammatory responses characterized by inflammatory cell infiltration are stronger in FUT1 KO mice than in WT mice.

I-4. UV-induced expression of pro-inflammatory cytokine, IL-1β, was significantly increased in FUT1 KO mice compared to WT mice, but the expression of anti-inflammatory cytokine, IL-10, did not show remarkable differences between FUT1 KO mice and WT mice

To compare the UV-induced inflammatory responses in FUT1 KO mice and WT mice, the levels of pro-inflammatory cytokine IL-1β **(Figure 5(a), 5(b))** and anti-inflammatory cytokine IL-10 **(Figure 5(c))** were examined by real-time RT-PCR. IL-1β has been known to act directly or indirectly on apoptosis inhibition, tumor progression and T cell development. IL-1β knockout and transgenic mouse models suggest a strong correlation of IL-1β expression with tumor development and progression (Voronov et al, 2006). However, unlike in human skin where keratinocytes themselves can secrete mature IL-1β, in murine skin, IL-1β production cannot be detected in keratinocytes following various inflammatory stimuli as well as UVR exposure *in vivo* (Nasti & Timares, 2012). Instead, infiltrating immune cells such as monocytes, macrophages and neutrophils or resident Langerhans cells are seen as the sources of IL-1β.

The expression level of IL-1β started to rise in a short time following UV irradiation and continued to increase until 48 hours post-irradiation. FUT1 deficiency in mice led to a more significant induction of IL-1β expression (1722±746%, 3089±1336%, 4520±2088% at 6 hours, 24 hours and 48 hours

after UVR exposure, respectively), when compared to that of the WT mice (561±220%, 638±240%, 1283±627% at 6 hours, 24 hours and 48 hours after UVR exposure, respectively). The levels of IL-1β were also determined using ELISA. At 6 hours after UV irradiation, the level of IL-1β was significantly higher for FUT1 KO mice (117.25±34 pg/ml) than for WT mice (72.57±14 pg/ml).

IL-10 inhibits the cytokine production of dendritic cells generated from CD34+ hematopoietic progenitor cells. Therefore, IL-10 plays an important role in the down regulation of cell-mediated immune response (Dittmar et al, 1999). There was a rapid increase in the level of IL-10 expression 6 hours after UV irradiation. Although the expression of IL-10 of FUT1 KO mice showed a higher increase at 6 hours and 24 hours after UVR exposure, compared to that of WT mice, the differences were not significant. These results indicate that severer UV-induced inflammatory responses associated with FUT1 deficiency in mice might be associated with an increase in pro-inflammatory cytokines.

Figure 4.

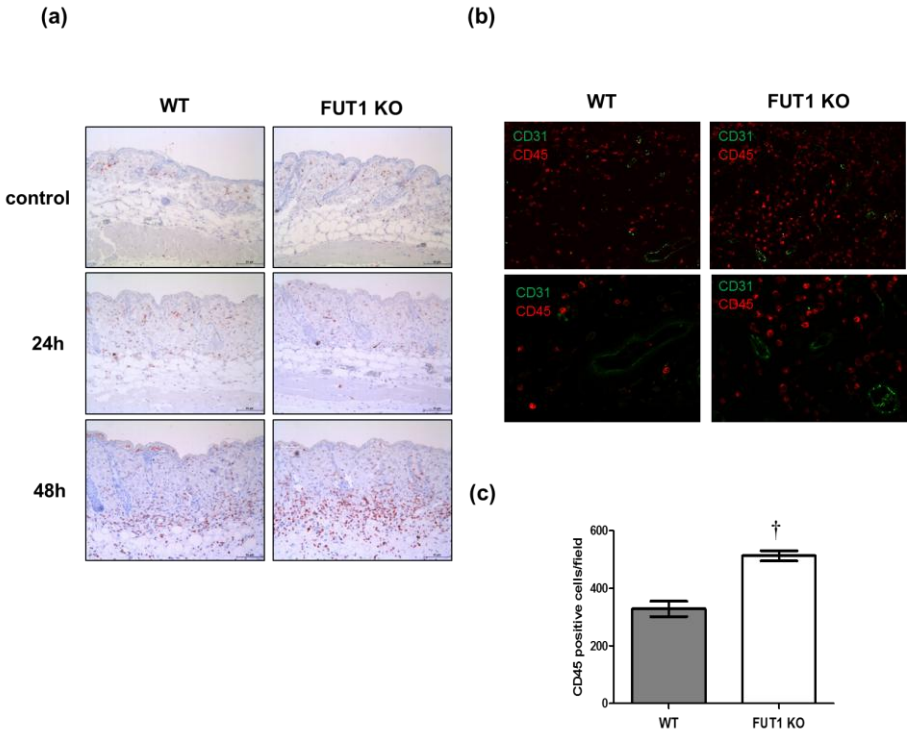

Figure 4. Higher levels of immune cell infiltration in UV-irradiated skin were observed in FUT1KO mice, in comparison with WT mice.

(a) The number of CD45-positive cells infiltrated in skin tissues were analyzed by immunohistochemical staining. (b) Immunofluorescence staining of CD31 and CD45 was also performed to visualize the adjacent blood vessels. (c) CD45-positive cells were more infiltrated in the skin of FUT1 KO mice than that of WT mice at 48 hours post-irradiation. (n=4 in each group. Error bars indicate standard error of the mean, †:$p<0.05$ vs. WT by Student's T test)

Figure 5.

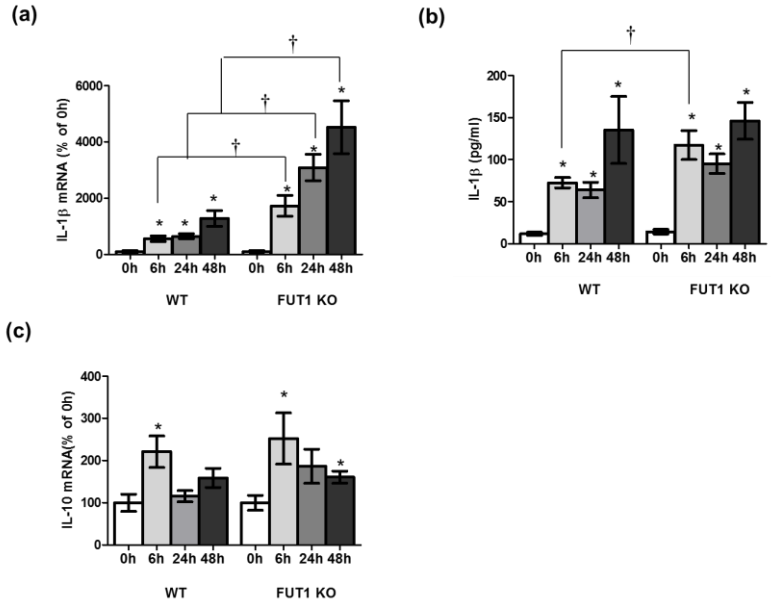

Figure 5. UV-induced expression of pro-inflammatory cytokine, IL-1β, was significantly increased in FUT1 KO mice compared to WT mice, but the expression of anti-inflammatory cytokine, IL-10, did not show remarkable differences between FUT1 KO mice and WT mice.

Following UV irradiation, skin was obtained at various time points (non-irradiated, 6hr, 24hr, 48hr). The expression level of IL-1β mRNA and protein were measured using **(a)** real-time RT-PCR and **(b)** MAP bead-based ELISA assay, respectively. **(c)** The expression level of IL-10, anti-inflammatory cytokines was also performed. (n=4 in each group. Error bars indicate standard error of the mean, *: $p<0.05$ vs. control, †:$p<0.05$ vs. WT by Student's T test)

I-5. The expression levels of IL-1 receptor antagonist (IL-1Ra) and NOD-like receptor family, pyrin domain containing 3 (NLRP3) did not show difference in FUT1 KO mice compared to WT mice

The expression levels of IL-1Ra and NLRP3 were examined. The levels of IL-1Ra, endogenous inhibitor which tightly regulates the activity of IL-1β, did not show a marked difference between FUT1 KO mice and WT mice **(Figure 6(a))**. In addition, the levels of NLRP3 which are involved in the formation of inflammasome and the activation of IL-1β were analyzed **(Figure 6(b))**, and there was no significant difference between FUT1 KO mice and WT mice.

I-6. UV-induced monocyte chemoattractant protein-1 (MCP-1) mRNA and chemokine (C-X-C motif) ligand 1 (CXCL1) mRNA were examined, but there was no difference between two genotypes

Infiltration of leukocytes is regulated by various chemokines. In mice, CXCL1, belonging to the CXC chemokine family (Haskill et al, 1990) acts as a specific chemoattractant for neutrophils by binding to its receptor CXCR2 (Smith et al, 2004). Murine endothelial cells start to express more CXCL1 in

response to cytokines, leading to the infiltration of neutrophils to the site of inflammation.

Endothelial cells, smooth muscle cells, and fibroblasts secrete MCP-1 in response to many physiological stimuli (Strieter et al, 1989). MCP-1 attracts monocytes, memory T lymphocytes, and NK cells *in vitro*. Several transgenic models of MCP-1 overexpression confirm that this chemokine can attract blood monocytes (Fuentes et al, 1995). It has also been reported that MCP-1 knock-out mice had severe defects in monocyte recruitment to the site of inflammation (Lu et al, 1998).

The expression levels of CXCL-1 and MCP-1 were measured in UV-irradiated FUT1 KO mice and wild type mice. The production of CXCL-1 **(Figure 7(a))** and MCP-1 **(Figure 7(b))** appeared to be significantly increased by UV irradiation in a short duration of time, 6 hours post-irradiation. However, the differences between FUT1 KO mice and wild type mice were not significant albeit the expression of MCP-1 showed a higher tendency in FUT1 KO mice. These results indicate that FUT1 deficiency is not essential to the UV-induced release of some chemokines such as CXCL-1 and MCP-1.

Figure 6.

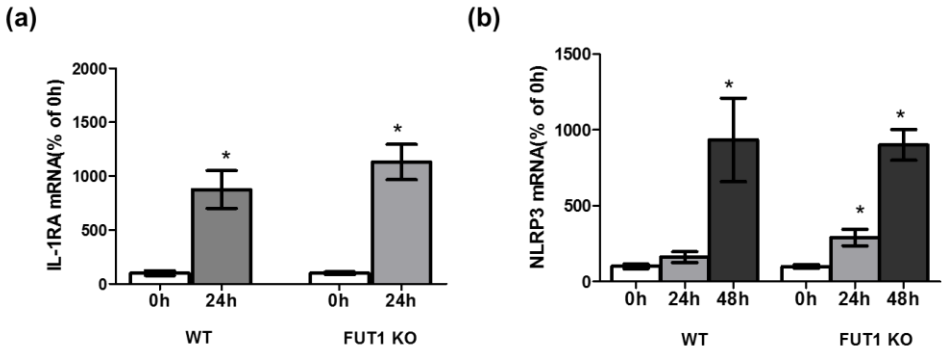

Figure 6. The expression levels of IL-1 receptor antagonist (IL-1Ra) and NOD-like receptor family, pyrin domain containing 3 (NLRP3) did not show difference in FUT1 KO mice compared to WT mice.

(a) IL-1Ra mRNA level at 24 hours post-irradiation was measured by using real-time RT-PCR. UV irradiation significantly increase the level of IL-1Ra, however, there was no remarkable difference between WT and FUT1 KO. (b) The mRNA level of NLRP3, involved in inflammasome formation, also did not show any difference between two genotypes at each time point (24hr, 48hr). (n=4 in each group. Error bars indicate standard error of the mean, *: $p<0.05$ vs. control by Student's T test)

Figure 7.

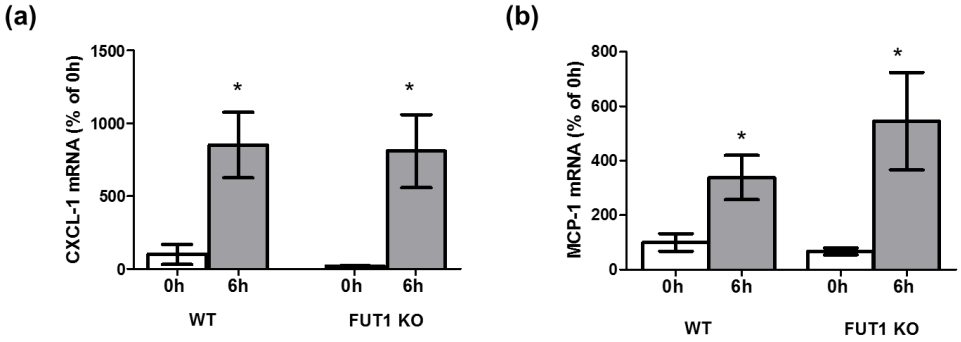

Figure 7. UV-induced monocyte chemoattractant protein-1 (MCP-1) mRNA and chemokine (C-X-C motif) ligand 1 (CXCL1) mRNA were examined, but there was no difference between two genotypes.

Proteins realated with immune cell chemotaxis were examined at 6 hours post-irradiation time point. (a) The mRNA level of CXCL-1 was measured using real-time RT-PCR. (b) MCP-1 was also examined in mRNA level. (n=3~4 in each group. Error bars indicate standard error of the mean, *: $p<0.05$ vs. control by Student's T test)

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I-7. UV-induced vascular endothelial growth factor (VEGF) showed no remarkable difference in FUT1 KO mice compared to wild type mice

FUT1 is reported to be involved in cell adhesion and angiogenesis, specifically in cancer cells. In human bladder cancer cells, FUT1 up-regulates fucosylation of β1-integrin, resulting in the activation of this protein in human bladder cancer (Lu et al, 2014).

VEGF is a key regulator of angiogenesis, and its expression is regulated by a variety of external factors that exert an angiogenic or anti-angiogenic effect. Interleukin 1β (IL-1β) is one of the various factors that can potentiate VEGF production (Li et al, 1995). VEGF, a highly specific mitogen for vascular endothelial cells, is also known to be derived in human keratinocytes via the MAPK/ERK kinase–ERK1/2 (extracellular signal-regulated kinase 1/2) pathway following UV exposure (Kim et al, 2006).

Therefore, UV-induced production of VEGF was examined in wild type mice and FUT1 KO mice **(Figure 8)**. The expression of VEGF slightly decreased at an early time point and subsequently increased 24 hours after UV irradiation. However, there was no significant difference between the two genotypes.

I-8. UV-induced Intercellular adhesion molecule-1 (ICAM-1) of FUT1 KO mice significantly decreased 24 hours after UV irradiation than that of wild type mice

ICAM-1, a cell adhesion molecule, which plays an important role in the generation of the epidermal inflammatory cell infiltration via leukocyte-keratinocyte interactions, has been accounted to increase by UV irradiation 48 hours after UV irradiation in human keratinocytes (Krutmann et al, 1992).

α-1,2-linked fucosylated proteins are highly expressed in rheumatoid arthritis synovial tissue when compared to the non-lesion tissue. It has been found that FUT1 in rheumatoid arthritis increases leukocyte cell adhesion (Isozaki et al, 2014).

Therefore, UV-induced ICAM-1 expression was studied to examine whether there are differences between FUT1 KO and WT mice **(Figure 9)**. The level of ICAM-1 was increased in 6 hours post-irradiation, and afterwards, it appeared to gradually decline. It was found that the expression level of ICAM-1 was significantly increased at the 24-hour point in FUT1 KO mice when compared to that of WT mice. These findings suggest that FUT1 deficiency may be involved in sustained upregulation of ICAM-1 when irradiated by UV.

Figure 8.

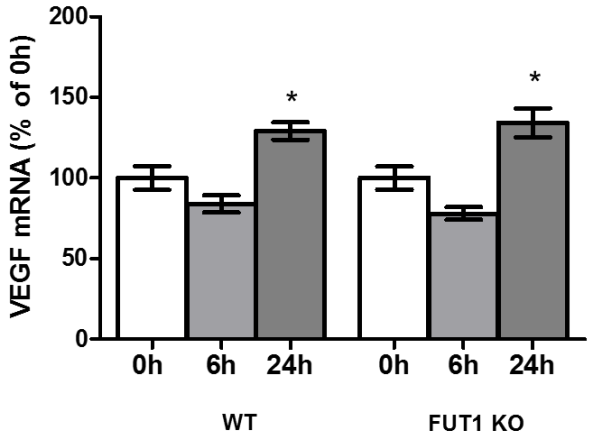

Figure 8. UV-induced vascular endothelial growth factor (VEGF) showed no remarkable difference in FUT1 KO mice compared to WT mice.

The mRNA level of VEGF, a key regulator of angiogenesis was examined at 6 and 24 hours after UV irradiation. (n=3~4 in each group. Error bars indicate standard error of the mean, *: $p<0.05$ vs. control by Student's T test)

Figure 9.

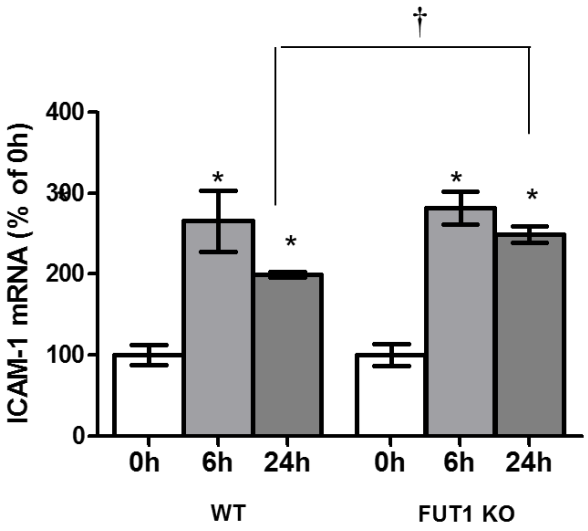

Figure 9. UV-induced Intercellular adhesion molecule-1 (ICAM-1) of FUT1 KO mice significantly decreased 24 hours after UV irradiation than that of WT mice

UV-induced ICAM-1expression level in WT and FUT1 KO mice was determined by real-time RT-PCR. (n=3~4 in each group. Error bars indicate standard error of the mean, *: $p<0.05$ vs. control, †:$p<0.05$ vs. WT by Student's T test)

Part II. Oxazolone-induced inflammatory responses in FUT1 KO mice

II-1. FUT1 KO mice showed more immune cell infiltration 48 hours after challenge. The ear thickness showed a significant increase after oxazolone challenge but there was not significant difference beteween FUT1 KO and wild type mice.

The ear thickness was measured **(Figure10(a))** with a digital caliper at indicated time points (18, 24, 42, 48 hours following challenge) and the ear punch biopsy were performed at 18 and 48 hours after challenge. The ear thickness started to increase and maintained an upward trend until 24 hours after oxazolone application. After that point, ear thickness were maintained until 42 hours following challenge. Oxazolone-induced allergic contact dermatitis model was histologically examined. Immue cell infiltration and epidermal thickening were analyzed by H&E staining **(Figure10(b))**. FUT1 KO mice showed more immune cell infiltration 48 hours after challenge. There was no remarkable difference in epidermal thicknening.

II-2. The expression level of inflammatory cytokines showed a tendency to higher 18 hours after challenge in FUT1 KO mice.

The inflammatory cytokines, IL-1β **(Figure 11(a))** and IL-10 **(Figure 11(b))** were examined in FUT1 KO and wild type mice. The expression level of these two cytokines were highest at 18 hours after oxazolone challenge. The expression level of inflammatory cytokines showed a tendency to higher 18 hours after challenge in FUT1 KO mice. Especially, IL-10 expression of FUT1 KO at 18 hours after challenge was significantly higher than that of wild type counterparts.

Figure 10.

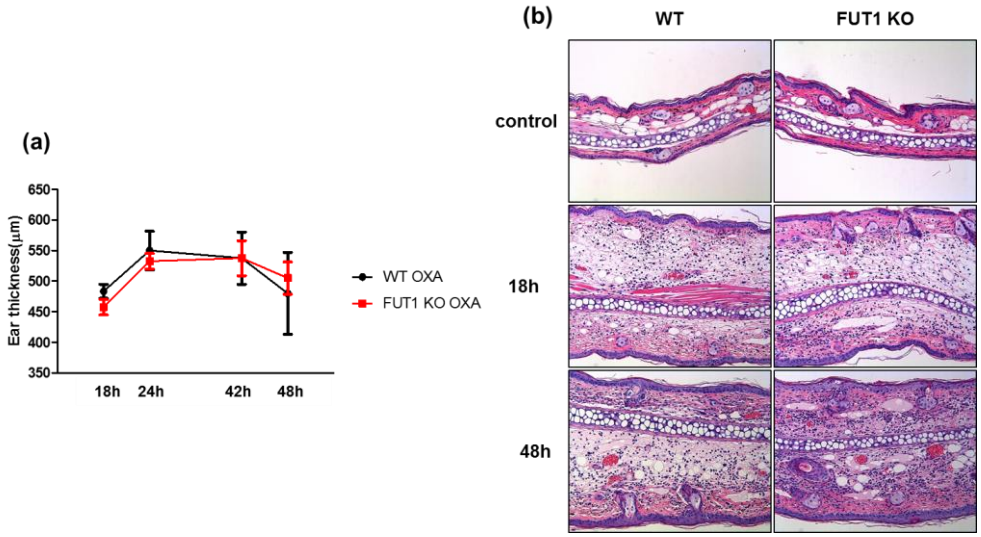

Figure 10. FUT1 KO mice showed more immune cell infiltration 48 hours after challenge. The ear thickness showed a significant increase after oxazolone challenge but there was not significant difference beteween FUT1 KO and wild type mice.

(a) Ear thickness was measured at 18, 24, 42,48 hours following challenge. (b) Histologicaly analysis were performed by H&E staining. Until 48 hours after challenge, epidermal thickening were gradually occurred. 48 hours after challenge, more immune cells were infiltrated into skin tissue of FUT1 KO compared to wild type mice.

Figure 11.

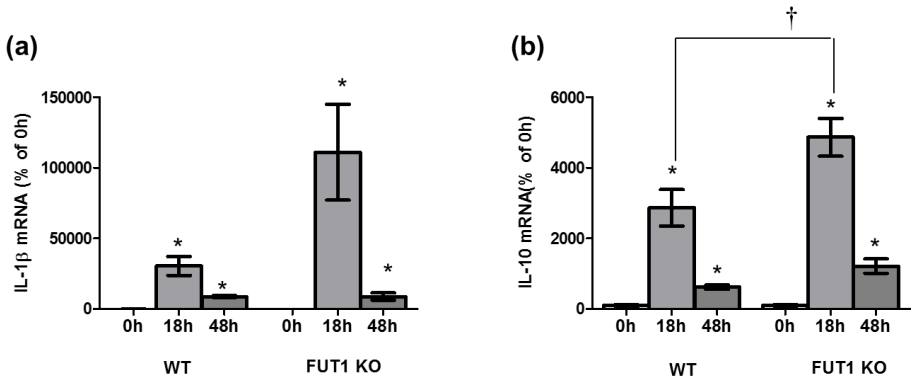

Figure 11. The expression level of inflammatory cytokines showed a tendency to higher 18 hours after challenge in FUT1 KO mice.

Oxazolone-induced inflammatory cytokines were examined by real time RT-PCR. (a) pro-inflammatory cytokine IL-1β and (b) anti-inflammatory cytokine IL-10 (n=3~4 in each group. Error bars indicate standard error of the mean, *: $p<0.05$ vs. control, †:$p<0.05$ vs. WT by Student's T test).

Part III. TPA-induced inflammatory responses in FUT1 KO mice

III-1. The ear thickness and the weight of ear biopsies showed a significant increase when 12-O-tetradecanoylphorbol 13-acetate (TPA) was applied, but there was no significant difference between FUT1 KO mice and wild type mice

The ear thickness was measured **(Figure 12(a))** with a digital caliper at indicated time points (baseline, 4, 6, 12, 24, 48 hours following TPA application) and the ear punch biopsy was performed and specimens were weighed **(Figure 12(b))** at 24 and 48 hours after the TPA application. Ears treated with TPA showed a significant increase in the ear thickness when compared to those with vehicles. The ear thickness started to increase from 4 hour point and maintained an upward trend until 24 hours after TPA treatment, which then began to decrease.

TPA-induced ear thickening of FUT1 KO mice (728±61.3 μm and 584±47.2 μm at 24 and 48 hours after TPA treatment, respectively) seemed to be lower than that of WT mice (764±40.6 μm and 686±83.8 μm at 24 and 48 hours after TPA treatment, respectively). However, the differences were not significant.

The ear weight of FUT1 KO mice (24.6±4.17 mg and 17.7±3.24 mg at 24 and 48 hours after TPA treatment, respectively) appeared to be lower than that of WT mice (26.96±2.27 μg and 20.3±3.16 μg at 24 and 48 hours after TPA treatment, respectively), but the differences between two groups were not significant.

Histological changes induced by TPA treatment were analyzed by H&E staining **(Figure 12(c))**. Unlike the ear thickness and weight, which showed a decreasing trend since 24 hours after TPA treatment, epidermal thicknesses as well as immune cell accumulations appeared to continuously increase until 48 hours after TPA treatment.

Figure 12.

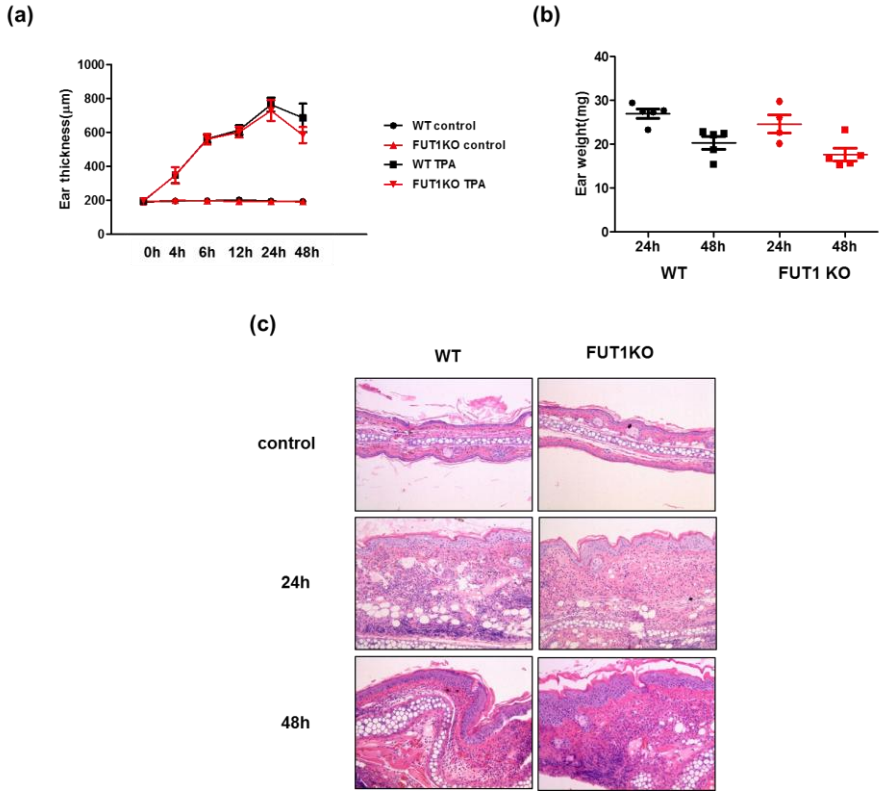

Figure 12. The ear thickness and the weight of ear biopsies showed a significant increase when 12-O-tetradecanoylphorbol 13-acetate (TPA) was applied, but there was no significant difference between FUT1 KO mice and WT mice.

TPA-induced skin inflammation was histologically analyzed. (a) The ear thickness of wild type and FUT1 KO mice was measured by a digital caliper at

0h, 4hr, 6hr, 12hr, 24hr and 48hr after TPA (0.01%) treatment. (b) The weight of ear specimen was measured at 24hr and 48hr following TPA treatment. (c) Histological evaluation using H&E staining indicated that unlike the ear thickness and weight, which appeared to decrease since 24 hours after TPA treatment, epidermal thicknesses as well as immune cell accumulations appeared to continuously increase until 48 hours after TPA treatment. (n=4~5 in each group)

III-2. TPA-induced ICAM-1 expression was increased in FUT1 KO mice, compared to WT mice

Previously, our group found that the expression of IFN-γ-induced ICAM-1 is significantly decreased in HaCaT keratinocytes transfected with FUT1 siRNA as compared with those transfected with scramble siRNA (unpublished observation). Besides, FUT1 knock-down in synovial fibroblasts showed a decrease in the expression of ICAM-1 and VCAM-1, which were known to be induced by TNF-α (Isozaki et al, 2014). These results suggest that FUT1 deficiency may reduce levels of cell adhesion molecules caused by the stimulation of cytokines, such as IFNγ and TNF-α.

Therefore, the expression level of ICAM-1 induced by TPA treatment was examined by immunohistochemical staining **(Figure 13)**. The TPA-induced ICAM-1 in epidermis was appeared to be decreased in FUT1 KO mice than in wild type mice, in agreement with the former findings *in vitro*. These results suggest that FUT1 deficiency may reduce TPA-induced inflammatory responses.

Figure 13.

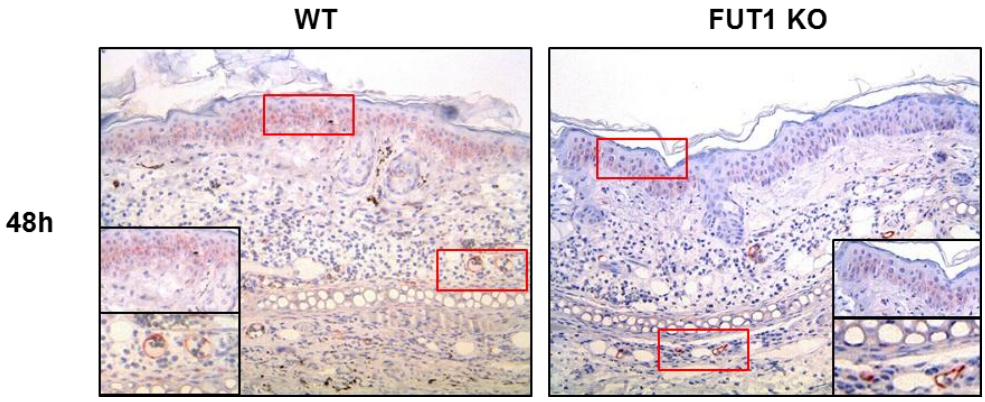

Figure 13. TPA-induced ICAM-1 expression was increased in FUT1 KO mice, compared to WT mice.

The expression level of ICAM-1 induced by TPA was examined by immunohistochemical staining 48hr after treatment. Immonoreactivities to ICAM-1 in epidermis were decreased in FUT1 KO mice than in WT mice. The expression of ICAM-1 was gradually increased from 24hr post-treatment to 48hr post-treatment and the difference between two genotypes was detected in 48hr time point. (n=4~5 in each group)

Discussion

α-1,2-fucosyltransferase 1 (FUT1) is the essential enzyme in synthesizing ABH blood group antigens, which are present in various tissues including skin as well as on human erythrocytes. Previous studies on the roles of ABH blood group antigens in tissues showed that FUT1 is over-expressed or under-expressed in several carcinomas, unveiling quite complex tissue-specific expression patterns of ABH antigen. Moreover, FUT1 has also been elucidated to mediate angiogenesis and inflammatory cell adhesion.

The mice with FUT1 deficiency was used at first to study about blastocyst adhesion required for murine implantation *in vivo* (Domino et al, 2001). During the rodent reproductive cycle, α-1,2-fucosylated glycan expression by the uterine epithelium is dynamically regulated in concert with hormonal changes, and several papers suggested α-1,2-fucosylated moieties might have a role in endometrial receptivity for blastocyst implantation (Kimber & Lindenberg, 1990). It was found that attachment between epithelia and blastocyst was dependent on α-1,2-fucosylated glycans (Lindenberg et al, 1988). To determine directly if cell surface α-1,2-fucosylated glycans are required for fertility, Domino et al. generated strains of mice that are deficient in genes encoding FUT1 and FUT2. Commercially available P1 genomic library was screened by specific PCR primers and targeting vectors were

derived from the β-galactoside-containing nucleus-localizing plasmid pnlacF. ES cells were transfected with the resulting targeting vector by electrophoration. Mice with induced homozygously null mutations in either the *FUT2* locus or the *FUT1* locus were viable and showed deficiency in the cell surface α-1,2-fucosylated glycans expressed by wild type mice. Both FUT1 null mice and FUT2 null mice, however, were fertile, indicating that α(1,2)fucosylated glycans play nonessential roles in blastocyst implantation.

Here, I investigated the role of FUT1 in skin inflammatory responses by using mice with FUT1 deficiency. To examine the different aspects of skin inflammation, both UV irradiation and contact dermatitis model were employed to induce skin inflammatory responses.

First I found that CD45-positive cells were more infiltrated in the skin of FUT1 KO mice than that of wild type mice at 48 hours post-UV irradiation **(Figure 4(c)).** CD45 is one of the most abundant leukocyte transmembrane glycoprotein known as leukocyte common antigen and is expressed on the surface of all hematopoietic lineage cells and their precursors. That is, skin inflammatory responses in FUT1 KO mice were severer than wild type counterparts. Although skin fold thickness, epidermal and dermal thickness as shown in H&E staining did not show a remarkable difference between two genotypes **(Figure 2)**, FUT1 deficiency would, at least partly, contribute to aggravated inflammatory responses induced by UV irradiation.

Pro-inflammatory cytokines, IL-1β were also increased in FUT1 KO mice compared to wild type mice **(Figure 5(a), 5(b))** In human skin, keratinocytes constitutively produce low levels of pro-IL-1β and pro-IL-18 which are accumulated in the cell cytoplasm. Keratinocytes require stimulation of both TLR and NLR receptors for secretion of mature IL-1β and IL-18 (Nasti & Timares, 2012). However, in murine skin, IL-1β production cannot be detected in keratinocytes following various inflammatory stimuli as well as UVR exposure *in vivo* (Nasti & Timares, 2012). In my experiments using mouse primary keratinocytes, similarly, the expression of IL-1β was not detected in both control cells and UV-irradiated cells (data were not shown). Instead, the sources of IL-1β are infiltrating bone marrow-derived cells (myeloid cell subsets - macrophages, monocytes, dendritic cells) and resident Langerhans cells (LCs). This has been found in the study which used bone-marrow chimeras of wild type and IL-1β−/− mice (Shornick et al, 2001). So the increased level of IL-1β in FUT1 KO mice seems to be related with the upregulation of CD45 positive cells which infiltrated to skin after UV irradiation, rather than directly influences of FUT1 deficiency in epidermal tissues. However, how FUT1 deficiency could attract more inflammatory cells to skin remains to be elucidated. Further investigations of the relationships between the fucosylation moieties formed by FUT1 and the chemotaxis events of CD45-positive cells are needed.

For immune cell infiltration from blood vessels to cutaneous tissues, rolling, adhesion and extravasation are required. These steps are finely controlled by several adhesion molecules such as ICAM-1, VCAM-1, selectins. The carbohydrate moietoies made between membrane glycoprotein of immune cells and vascular endothelial cells are considered to have a important role for rolling and adhesion. Among several types of glycosylation affecting the carborhydrate moieties, blood group antigen sialyl Lewis x has essential role interacting with E-selectins on endothelial cells. Both sialyl Lewis x and H type 2 antigen are made from H precursor (Gal-AcGluNH). Different types of fucosyltransferases are involved in synthesis of these two antigens. For making H type 2 antigen, α(1,2)-linked fucose is required. Each fucosyltransferase can utilize specific substrate, synthesizing particular product. From this kind of characteristics, it can be possibly suggested that FUT1 dificiency might affect the amount of H precursor, resulting in increase of sialyl Lewis x. Consquently, cellular rolling and adhesion would be increased, explaining why FUT1 KO mice showed more CD45 positive cells infiltrated compared to wild type after UV irradiation. However, further studies should be conducted.

IL-1β regulates systemic and local responses to infection, injury and immunological challenge by generating fever, activating lymphocytes and promoting leukocyte transmigration into sites of injury or infection (Lamkanfi,

2011). The expression level of IL-1β is tightly controlled by various factors. IL-1β is produced as cytosolic precursors and requires cleavage by caspase-1 for fully activated and secreted (Gu et al, 1997). Inflammasomes are intracellular multiprotein complexes that mediate the proximity-induced autoactivation of caspase-1 (Lamkanfi, 2011). Inflammasome-mediated caspase-1 activation occurs in macrophages, dendritic cells, epithelial cells during bacterial, viral, fungal and parasitic infections. In mice, there are at least four types of inflammasome. NLR pyrin domain-containing 1B (NLRp1B), NLR CARD-containing 4 (NLRC4) and NLRP3 are commonly contain NLR proteins and the other type of inflammasome contains the HIN-200 protein absent in melanoma 2 (AIM2) (Lamkanfi, 2011).

To futher investigate the causes of increased mRNA and protein level of IL-1β, the expression level of NLRP3, known to be activated by UV irradiation (Harder et al, 2009; Lamkanfi, 2011), was examined **(Figure 6(b))**. However, there was no significant difference in FUT1 KO compared to WT. It is noteworthy that additional proteases, including caspase-8, myeloblastin called proteinase 3 and granzyme A, have been shown to convert pro-IL-1β into a biologically active form in several established mouse models (Irmler et al, 1995; Joosten et al, 2009; Maelfait et al, 2008). This indicates that caspase-1 is not always required for the maturation of IL-1β, and such redundancy in controlling IL-1β maturation might safeguard the host immune response

against viral and bacterial pathogens that target caspase-1 activation in inflammasomes (Kanneganti, 2010).

In oxazolone-induced inflammatory responses in FUT1 KO, more immune cells seemed to be infiltrated in cutaneous tissue considering the H&E staining results **(Figure 10(b))**. H&E staining cannot separate out the types of immune cells. Immunochemical staining for CD45 or other immune cell markers should be coducted for more exact results. Although the difference was not significant, the ear thickness of FUT1 KO were thicker than that of wild type on average **(Figure 10(a))**. These results suggest that FUT1 KO are more susceptible than wild type to ear edema induced by allergic contact dermatitis model. Inflammatory cytokines were also highly expressed in FUT1 KO mice **(Figure 11)**.

It was observed that the expression level of ICAM-1 was significantly increased in FUT1 KO mice compared with WT mice, when it was induced by UV irradiation. On the other hand, the ICAM-1 induced by TPA treatment was less detected in FUT1 KO mice, compared to WT mice **(Figure 13)**. However, the mRNA levels of inflammatory cytokines or chemokines in this tissues were not checked yet. More studies should be still done to confirm this results.

The molecular mechanisms of skin inflammation induced by UV irradiation are complex and have not been completely understanded. Cell survival,

apoptosis, migration, proliferation are regulated by UV irradiation (Rosette & Karin, 1996). UV-irradiation itself can result in multimerization of cell-surface receptors, which can activate the Jun N-terminal kinase (JNK) signalling pathway. A wide array of molecules has been eluciated to be activated, altered or produced by UVR stimulation. These molecules include receptors, DNA/RNA, reactive oxygen species (ROS), cytokines, adhesion molecules, matrix metalloproteinases (MMP), cis-urocanic acid. DNA damage may also be involved in signalling pathways following UV irradiation (Bender et al, 1998). Not only that, regulation of cytokine profiles is important in UV-induced skin inflammation (Clydesdale et al, 2001). Epidermal keratinocytes and dermal cells produce a wide range of pro and anti-inflammatory cytokines, such as IL-1, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15 and TNF-α.

The thing that differentiates hapten-induced allergic contact dermatitis(ACD) model from UV or TPA induced inflammatatation is that its response is antigen-specific. Following antigen-nonspecific and general responses, antigen-spefic inflammation are occurred. Subsets of immune cells, such as regulatory T cells (Tregs) and CD4+ T-helper 17 (Th17) cells have been implicated from studies on ACD model. Not only that, but also the discovery of Langerin-positive dermal dendritic cells (DCs) questioned the relevance of epidermal Langerhans cells (LCs) as key antigenpresenting cells in cutaneous immune responses (Honda et al, 2013).

Chemical irritants, such as TPA or DNCB induce the skin inflammation mainly via pro-inflammatory cytokine TNF-α (Kupper, 1990). After the exposure of irritants on the skin, epidermal cells release pro-inflammatory cytokines leading to vasodilation and leukocyte infiltration. TNF-α induces the expression of various adhesion molecules as well as chemokines. Adhesion molecules induced by TNF-α includes vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin (Kupper, 1990). Adhesion molecules and chemokines attracts leukocytes such as T lymphocytes, neutrophils and infiltrated leukocytes which mediate inflammatory responses (Han et al, 2007).

In conclusion, these findings suggest that FUT1 have a role in the cutaneous inflammation induced by UV irradiation and TPA treatment. It has been previously found FUT1 and its fucosylated moieties have abilities to regulate many physiological processes such as cell adhesion, rolling and migration. In this study, mice with FUT1 deficiency were firstly tested to examine whether this fucosyltransferase is involved in the process of immune cell migration and cutaneous infiltration. Although both UV and chemicals such as oxazolone and TPA could induce inflammatory responses to FUT1 KO mice, different underlying mechanisms of each stimulation causes various results, showing the possibilities of various roles of FUT1 in skin inflammation. It is also worth considering that H type 2 antigens, synthesized by FUT1, are strongly

expressed in blood vessels and epidermis. This finding supports that extravasation and immune cell infiltration, to some extent, might be regulated by FUT1 and ABH antigens. A structural analysis as well as underlying mechanism of α-1,2-fucosylated glycan and cell surface adhesion molecules should be elucidated in future studies.

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