NSV. To find whether these promoter SNPs affect transcription factors, the online program AliBaba 2.1 (http://www.gene-regulation.com/pub/programs/alibaba2) was used. At the rs9274552 SNP, the A-containing sequences bind with C/EBPa1p transcription factor, but C/EBPa1p disappears in the C-containing sequences. At the rs9274579 SNP site, the A-containing sequences interact with Oct-1, but Oct-1 transcription factor disappear in the G-containing sequences. Assuming the change of transcription factors according to variants of SNPs, these promoter SNPs may influence gene and protein expression of HLA-DQB1.

Our results indicate that the HLA-DQB1 promoter polymorphism (rs9274552) may increase susceptibility to NSV in Korean population along with genes previously confirmed to play a role in polygenic susceptibility to NSV. Further studies are needed to clarify the exact role of HLA-DQB1 on NSV pathogenesis.

Conflict of interest

The authors have no conflict of interest to declare.

Funding sources

None.

References

[1] P.R. Fain, S.R. Babu, D.C. Bennett, R.A. Spritz, HLA class II haplotype DRB1*04-DQB1*0301 contributes to risk of familial generalized vitiligo and early disease onset, Pigm. Cell Res. 19 (2006) 51–57.
[2] K. Oyarbide-Valencia, J.G. van den Boorn, C.J. Denman, M. Li, J.M. Carlson, C. Hernandez, et al., Therapeutic implications of autoimmune vitiligo T cells, Autoimmun. Rev. 5 (2006) 486–492.
[3] S. Glisic, M. Klinker, J. Waukau, P. Jailwala, S. Jana, J. Basken, et al., Genetic association of HLA DQB1 with CD4+CD25+(high) T-cell apoptosis in type 1 diabetes, Genes Immun. 10 (2009) 334–340.
[4] S. Yang, J.Y. Wang, M. Gao, H.S. Liu, L.D. Sun, P.P. He, et al., Association of HLA-DQA1 and DQB1 genes with vitiligo in Chinese Hans, Int. J. Dermatol. 44 (2005) 1022–1027.
[5] G. Orecchia, L. Perfetti, P. Malagoli, F. Borghini, Y. Kipervarg, Vitiligo is associated with a significant increase in HLA-A30, Cw6 and DQw3 and a decrease in C4AQ0 in northern Italian patients, Dermatology 185 (1992) 123–127.
[6] M. Buc, H. Fazekasová, E. Cechová, E. Hegyi, K. Kolibásová, S. Ferencík, Occurrence rates of HLA-DRB1, HLA-DQB1, and HLA-DPB1 alleles in patients suffering from vitiligo, Eur. J. Dermatol. 8 (1998) 13–15.
[7] M. Zamani, M. Spaepen, S.S. Sghar, C. Huang, W. Westerhof, L. Nieuweboer-Krobotova, et al., Linkage and association of HLA class II genes with vitiligo in a Dutch population, Br. J. Dermatol. 145 (2001) 90–94.

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Received 22 September 2015
Received in revised form 28 December 2015
Accepted 14 January 2016

http://dx.doi.org/10.1016/j.jdermsci.2016.01.006

Letter to the Editor

Immunosuppressive effect of adipose-derived stromal cells on imiquimod-induced psoriasis in mice

Letter to the Editor,

Psoriasis is one of the most common immune-mediated chronic, inflammatory skin diseases characterized by hyperproliferative keratinocytes and infiltration of T cells, dendritic cells, macrophages and neutrophils [1]. Especially, T helper 17 (Th17) cells and interleukin (IL)-17A, IL-22, and IL-23, known as Th17-related cytokines, play roles in the pathogenesis of psoriasis. Moreover, tumor necrosis factor (TNF) maintains an inflammatory loop in psoriatic lesions and promotes the increased expression of IL-17A, IL-21, and IL-22 in Th17 cells [2]. Imiquimod (IMQ)-induced psoriasis-like mice models feature lesions clinically and pathologically similar to human psoriasis lesion [3].

In 2001, Zuk et al. reported that stem cells could be isolated from human adipose tissue [4]. Many clinical studies have incorporated adipose-derived stromal cells (ASCs) for the potential therapeutic use in various diseases, although the exact mechanisms underlying their functions remain to be investigated. Herein, we focused on the regulatory and inhibitory effects of ASCs in the psoriatic skin and attempted to discover clues toward improving psoriasis.

We applied IMQ cream on the shaved dorsal skin for 5 consecutive days. On days 0, 2, and 4, either ASCs or PBS were injected into the intradermal dorsal areas (Fig. 1a). On day 6 (D6), we identified the location of surviving ASCs on the dorsal skin using in vivo imaging system (Fig. 1b). Three days after IMQ application, the dorsal skin treated with both IMQ and PBS began to display signs of erythema, scaling and thickening. These symptoms of inflammation increased in severity throughout the experiment (Fig. 1c). However, the dorsal skin co-treated with IMQ and ASCs exhibited only slight erythema, scaling and thickening on D6, indicating that the ASCs inhibited IMQ-induced inflammatory changes in murine skin. In hematoxylin and eosin staining, the cell cluster was located in the dermis of ASC-injected mice (Fig. 1d). Furthermore, we disclosed that these cells are consistent with fluorescence-labeled ASCs (Fig. 1d). A histological examination revealed epidermal hyperkeratosis and parakeratosis in the skin of mice treated with IMQ and PBS (Fig. 1e). On the other hand, the skin of ASC-injected mice exhibited no significant psoriatic findings. Moreover, ASC-injected mice had a significantly thinner epidermis, compared with PBS-injected mice (Fig. 1f). These

Abbreviations: ASC, adipose-derived stromal cell; IMQ, imiquimod; FBs, fibroblasts; MSC, mesenchymal stem cell; BM, bone marrow.

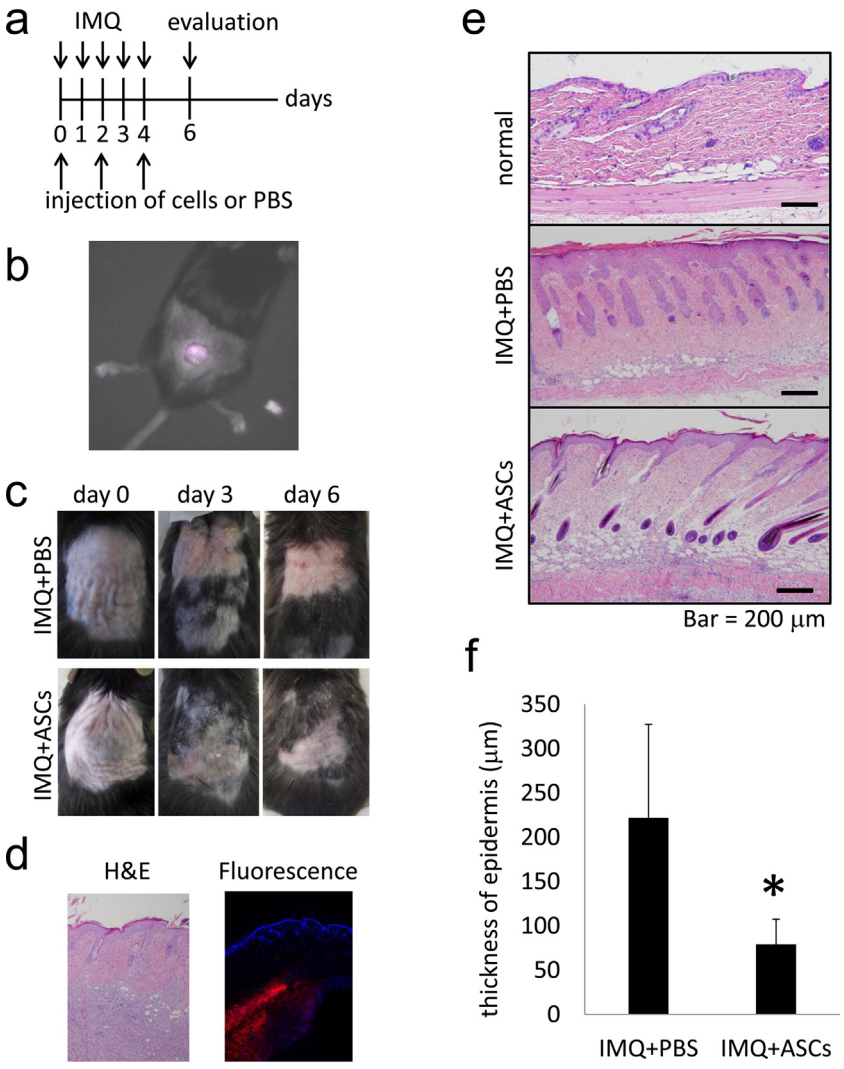

Fig. 1. The immunosuppressive effect of murine adipose-derived stromal cells (ASCs) on imiquimod (IMQ)-induced psoriatic inflammation. (a) IMQ was applied to shaved dorsal skin of mice for 5 consecutive days. On days 0, 2, and 4, either 1×10^6 cells or PBS was intradermally injected into the IMQ-treated skin. On D6, mice were sacrificed for skin and blood sample collection. (b) In vivo imaging of injected living ASCs (Fig. 1b). (c) Consecutive IMQ application clearly induced severe erythema and scaling, similar to that observed in psoriatic skin. In contrast, skin co-treated with IMQ and ASCs exhibited only slight scaling and erythema. (d and e) Samples of IMQ-treated dorsal skin were taken on D6 and were observed by microscope. Each representative section is shown ($100 \times$ magnification, scale bar = 200 μm). (f) Comparison of the epidermal thickness in murine dorsal skin samples following co-treatment with IMQ and either ASCs or PBS. The ASC-injected epidermis was significantly thinner than the PBS-injected epidermis. Data are presented as mean ± standard deviations ($n = 5$/group, $^*p < 0.05$).

findings suggested that ASCs could strongly inhibit not only the inflammation but also psoriatic scaling and erythema.

To elucidate the immunosuppressive effects of intradermally injected ASCs in IMQ-induced psoriatic mice, spleens were harvested and measured on D6. In addition to the mice treated with PBS or ASCs, another group of mice received intradermal injections of FBs, which are similar to ASCs in morphology and appearance, into the IMQ-treated areas of skin. All IMQ-treated mice exhibited splenomegaly, compared with normal mice (Fig. 2a and b). However, intradermal ASC injection had a tendency to reduce the weights and sizes of spleens in IMQ-applied mice.

To examine the systemic effects of ASCs in IMQ-induced psoriatic mice, we analyzed the levels of the IL-17A, TNF-α, IL-23, and IL-6 using ELISA. The serum levels of these cytokines were significantly lower in ASCs-injected mice than in PBS-injected mice ($p < 0.01$; Fig. 2c). This indicates that the direct administration of ASCs to the intradermal skin inhibits systemic IMQ-induced inflammation. Furthermore, we quantified IL-17A and TNF-α mRNA expression in skin lesions and spleens from IMQ-applied mice co-treated with either type of cells or PBS. The splenic levels of IL-17A mRNA were significantly lower in ASC-injected mice than in FB-injected mice ($p < 0.01$), although there was no difference when compared with PBS-injected mice (Fig. 2d). We also did not find significant differences in TNF-α mRNA expression among the groups. Conversely, IL-17A and TNF-α were significantly upregulated in the skin lesions of PBS- and FB-treated mice, compared with ASC-injected mice ($p < 0.01$)(Fig. 2d). These results indicate that ASCs inhibit the production of Th17-associated cytokines, such as IL-17A and TNF-α, and minimize the psoriatic skin changes induced by IMQ. These results demonstrate the potential of ASCs as strong immunosuppressive mediators in psoriasis.

Mesenchymal stem cells (MSCs) are immunoregulatory and multipotent progenitor cells that can be easily isolated and expanded from the bone marrow (BM), umbilical cord, and other tissues. MSCs and ASCs are thought to possess similar characteristics in terms of cell proliferation, anti-inflammatory effects, and regeneration-promoting factor secretion. In our study, IMQ-induced skin lesions treated with murine ASCs had normal levels of IL-17A and TNF-α mRNA, in contrast to untreated IMQ-induced skin lesions, suggesting that murine ASCs could locally inhibit the

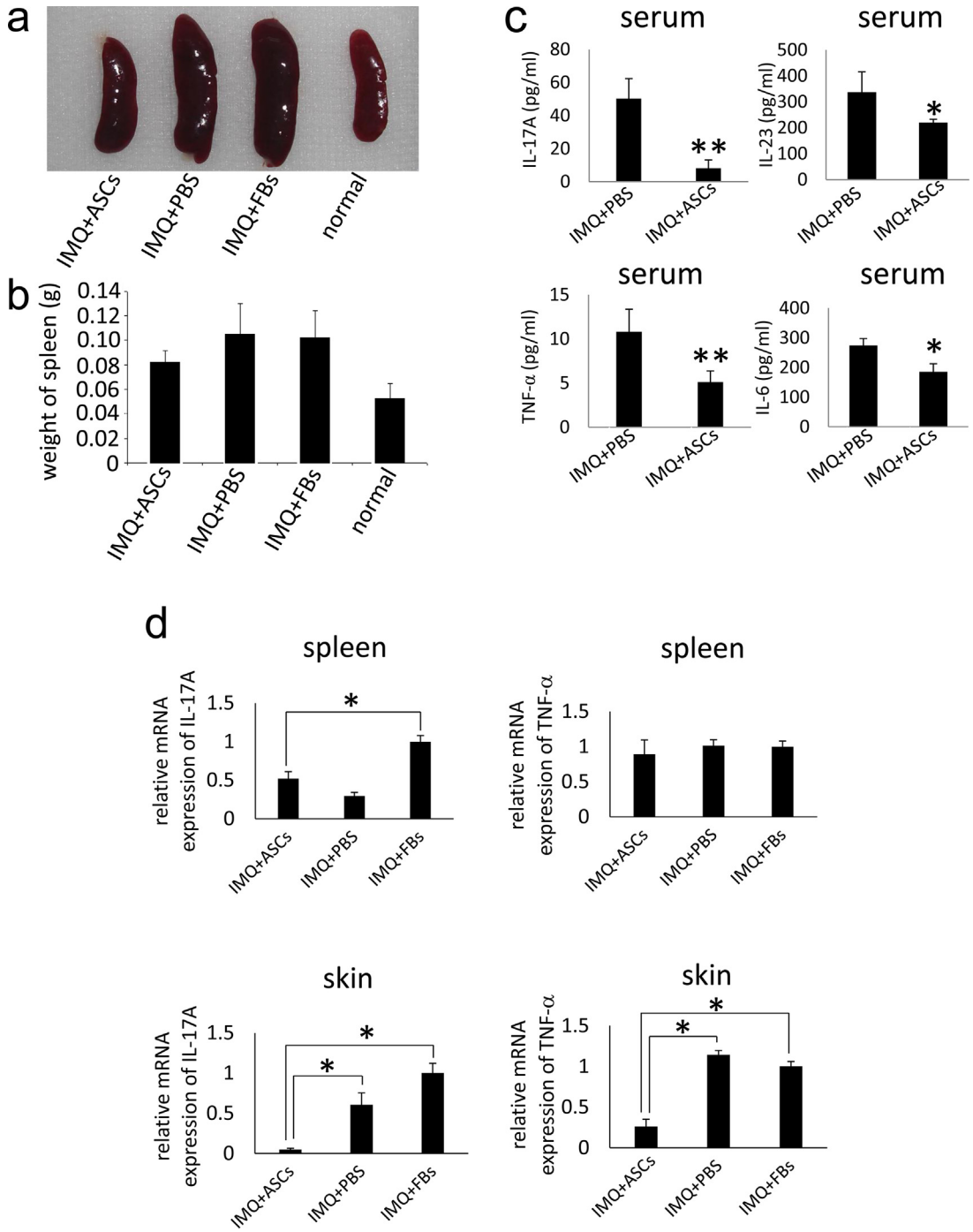

Fig. 2. (a)and (b) Inhibitory effect of adipose-derived stromal cells (ASCs) on imiquimod (IMQ)-induced splenomegaly. Both the weights and sizes of spleens in IMQ-applied mice were markedly reduced following intradermal injections of ASCs ($n = 5$/group). Representative images from each group are shown. (c) Downregulation of IMQ-induced interleukin (IL)-17A, tumor necrosis factor (TNF)-α, IL-23, IL-6 in response to ASC treatment. After sacrifice, the serum levels of inflammatory cytokines were measured by enzyme-linked immunosorbent assay. Serum levels of IL-17A, TNF-α, IL-23 and IL-6 were significantly lower in ASC-treated mice than in PBS-treated mice. Data are presented as means ± standard deviations ($n = 5$/group, $^{*}p < 0.05$, $^{**}p < 0.01$). (d) Inhibitory effect of ASCs on IMQ-induced IL-17A and TNF-α mRNA overexpression. RNA was isolated from spleens and IMQ-treated dorsal skin from mice treated with either cells or PBS and subjected to a quantitative reverse transcription-polymerase chain reaction analysis to determine the levels of IL-17A and TNF-α mRNA. The relative splenic levels of IL-17A mRNA expression were significantly lower in ASC-treated mice than in fibroblast (FB)-treated mice ($^{*}p < 0.05$). However, the relative expression levels of TNF-α mRNA were similar among the 3 groups. The relative skin expression levels of IL-17A and TNF-α mRNA were significantly lower in ASC-treated mice, compared to PBS- and FB-treated mice. Data are presented as means ± standard deviations ($n = 5$/group, $^{*}p < 0.05$).

inflammatory signals transmitted via Toll-like receptors 7/8 in IMQ-treated skin.

Recent studies have revealed that MSCs from human psoriatic lesions express high levels of inducible nitric oxide synthase and vascular endothelial growth factor, relative to non-lesional skin, suggesting that dermal MSCs from patients with psoriasis might play a role in the early development of psoriasis because of their pro-angiogenic potential [5]. Although ASCs and MSCs appear similar, high volumes of isolated ASCs functioned locally as immunosuppressive effectors when administered regionally. The suppressive effect of ASCs on local IL-17A overexpression might cut off the psoriatic cytokine network formed by Th17 cells and dermal MSCs. Other studies revealed that BM-MSCs strongly inhibit Th17 cell differentiation through the activation of suppressors of

cytokine signals 3 [6]. In our in vivo study, we found that local ASC application inhibited the IMQ-induced upregulation of IL-17A and TNF-α expression and maintained a clinically normal environment in murine skin. These results are consistent with those of a previous report [6], despite the use of different stromal cell resources.

Recent study reported that MSCs secrete TNF-α-stimulated gene/protein 6, which modulates the activation of the resident macrophages or decrease the downstream effects of the proinflammatory cytokines [7]. Furthermore, MSCs activate IL-10 production from macrophages, which suppresses inflammatory reactions by inhibiting both neutrophil infiltration and T-cell responses [8]. These imply that ASCs might similarly represent immunosuppressive effects via the secretion of these cytokines.

In this study, we have proven the potential clinical application of allogeneic or autogenic ASCs for the treatment of psoriasis through a process that differs considerably from existing biological therapies.

Funding

None.

Conflict of interests

The authors have no conflict of interest to declare.

Acknowledgments

The authors thank Ms. Yukiko Tamura, Yuka Toyomaki, and Ikuko Takahashi for their excellent technical assistance. This work was supported by KAKENHI (26461675).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2015.12.007.

References

[1] Y. Cai, C. Fleming, J. Yan, New insights of T cells in the pathogenesis of psoriasis, Cell Mol. Immunol. 9 (2012) 302–309.
[2] L. Grine, L. Dejager, C. Libert, R.E. Vandenbroucke, An inflammatory triangle in psoriasis: TNF, type I IFNs and IL-17, Cytokine Growth Factor Rev. 26 (2015) 25–33.
[3] L. van der Fits, S. Mourits, J.S. Voerman, M. Kant, L. Boon, J.D. Laman, et al., Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis, J. Immunol. 182 (2009) 5836–5845.
[4] P.A. Zuk, M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, et al., Multilineage cells from human adipose tissue: implications for cell-based therapies, Tissue Eng. 7 (2001) 211–228.
[5] M. Orciani, A. Campanati, E. Salvolini, G. Lucarini, G. Di Benedetto, A. Offidani, et al., The mesenchymal stem cell profile in psoriasis, Br. J. Dermatol. 165 (2011) 585–592.
[6] X. Liu, S. Ren, X. Qu, C. Ge, K. Cheng, R.C. Zhao, Mesenchymal stem cells inhibit Th17 cells differentiation via IFN-γ-mediated SOCS3 activation, Immunol. Res. 61 (2015) 219–229.
[7] E. Aikawa, R. Fujita, Y. Kikuchi, Y. Kaneda, K. Tamai, Systemic high-mobility group box 1 administration suppresses skin inflammation by inducing an accumulation of PDGFRα(+) mesenchymal cells from bone marrow, Sci. Rep. 5 (2015) 11008.
[8] D.J. Prockop, J.Y. Oh, Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation, Mol. Ther. 20 (2012) 14–20.

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Received 3 August 2015
Received in revised form 8 December 2015
Accepted 16 December 2015

http://dx.doi.org/10.1016/j.jdermsci.2015.12.007

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Inhibition of epidermal growth factor receptor induces tumor necrosis factor-α via activation of peroxisome proliferator-activated receptor-γ and nuclear factor-κB in sebocytes: A possible pathogenesis of papulopustular rash

Keywords
EGFR inhibitor
Papulopustular rash
TNFα
PPARγ
NF-κB
Differentiation

Epidermal growth factor receptor inhibitors (EGFRIs) are used to treat various cancers; however, they are associated with a high prevalence of cutaneous side effects. Papulopustular rash is the most frequent adverse side effect and, occasionally, necessitates dose adjustment or interruption of therapy.

Experimental blockade of epidermal growth factor receptor (EGFR) induces aberrant cytokine expression in keratinocytes, leading to skin inflammation [1,2]. EGFR is expressed by proliferating keratinocytes in sebaceous glands and in the epidermis [3]. Dysregulation of sebocytes may induce aberrant cytokine expression, leading to papulopustular rash. Here, we treated SEB-1-immortalized human sebocytes with two EGFRIs (erlotinib and gefitinib) and examined their effects on cytokine expression by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) (see "Supplemental information" for detailed methods).

EGFRI increased expression of *interleukin (IL)-5*, *IL-6*, *IL-12A*, *tumor necrosis factor-α (TNFα)*, *plasminogen activator inhibitor-1 (PAI-1)*, *Toll-like receptor (TLR)2*, and *TLR3* by more than 1.5-fold (Supplemental Fig. 1A). Of these, *TNFα* was expressed at higher levels in SEB-1 cells than in HaCaT keratinocytes or

Abbreviations: COX-2, cyclooxygenase-2; EGFR, epidermal growth factor receptor; EGFRI, epidermal growth factor receptor inhibitor; FAS, fatty acid synthase; IL, interleukin; MC5R, melanocortin 5 receptor; NF-κB, nuclear factor-κB; PAI-1, plasminogen activator inhibitor-1; PPARγ, peroxisome proliferator-activated receptor-γ; PPRE, peroxisome proliferator response element; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SREBP-1, sterol regulatory element-binding protein-1; TLR, toll-like receptor; TNFα, tumor necrosis factor-α.