Saudi Pharmaceutical Journal 25 (2017) 620–624

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Acitretin modulates HaCaT cells proliferation through STAT1- and STAT3-dependent signaling

Xuan Qin [a,b,1], Chunna Chen [a,1], Yuan Zhang [a,1], Li Zhang [a], Yijie Mei [a], Xinchun Long [b], Rui Tan [a], Wenli Liang [c,*], Ledong Sun [a,*]

[a] Department of Dermatology, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, China
[b] Department of Dermatology, The First People's Hospital of Chenzhou, Chenzhou 423000, China
[c] Nursing School of Guangdong Pharmaceutical University, Guangzhou 510318, China

ARTICLE INFO

Article history:
Available online 8 May 2017

Keywords:
Acitretin
HaCaT cells
STAT1
STAT3
SOCS1
SOCS3

ABSTRACT

Acitretin has been a valuable option for the treatment of psoriasis, however, the molecular events of acitretin leading to the normalization of keratinocytes differentiation on psoriasis patients have not been fully explored. To investigate whether there were certain relationship between keratinocytes proliferation and JAK/STAT signaling pathways in psoriasis, and how acitretin modulated the signaling pathways. HaCaT cells, an in vitro immortal human keratinocyte cell line, was chosen as a in vitro model of psoriasis. The small interfering RNA targeting STAT1 (siRNA-STAT1) and STAT3 (siRNA-STAT3) were subsequently transfected into the HaCaT cells which were treated with or without acitretin. We found that HaCaT cells proliferation and the expression of STAT1 or STAT3 were inhibited by acitretin, siRNA-STAT1 and siRNA-STAT3. Our experimental data shows that acitretin might inhibit HaCaT cells proliferation in psoriasis by decreasing the expression of STAT- and STAT3-dependent mechanism.

1. Introduction

Psoriasis is a chronic, common, and immune–mediated inflammatory skin disorder (Vassantachart et al., 2016; Torres et al., 2016). Although its pathogenesis remains unclear, psoriasis is now considered as a mixed Th1/Th17 cell-mediated autoimmune disease because the activated T lymphocytes release numerous cytokines, such as interferon-γ (IFN-γ) and IL-17, to stimulate the proliferation of keratinocytes and recruit neutrophils. The cytokines-activated keratinocytes can also express a broad array of cytokines, chemokines and membrane molecules in return that induce the recruitment and activated of T lymphocytes in the skin (Wang et al., 2015; Iftakhar et al., 2015; Kupetsky et al., 2013). In addition, those key cytokines including IFN-γ, IL-6, IL-8, IFN-α, IL-12 and IL-17 were up-regulated in psoriasis patients (Johnson-Huang et al., 2012; Martin et al., 2013; Zhao and Ashraf, 2016). Thus, the T cell/cytokines/keratinocytes network is formed and partake in the pathogenesis of psoriasis.

Those key cytokines of psoriasis such as IL-6, IL-17, IL-22 and INF-γ are mediated by JAK-STAT signaling pathway. STAT1 and STAT3 can be activated by IFN-γ and IL-6 signaling, respectively. Many researches have demonstrated that the JAK-STAT signaling pathway is involved in melanoma, atopic dermatitis and psoriasis (Luo et al., 2016; Liongue and Ward, 2013; Palanivel et al., 2014). Therefore, selective blockading the JAK-STAT signaling pathway could be a potential strategy for those common skin disorders. Acitretin plays an critical role in the treatment of psoriasis thanks to its non-immunosuppressive risks and its ability to complete a long-term response (Yine et al., 2015; Sarfraz et al., 2016; Carretero et al., 2013). So far, the effects of acitretin on JAK-STAT signaling pathway of keratinocytes have been rarely reported in literatures. In this study, we explored the relationship between keratinocytes proliferation and STAT1, STAT3 expression and how acitretin modulates the JAK/STAT signaling pathways.

* Corresponding authors.
E-mail address: Sunledong126@126.com (L. Sun).
[1] These authors contributed equally to this work.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2017.04.034
1319-0164/

2. Methodology

2.1. Cell culture

HaCaT cells was obtained from institute of Biochemistry and Cell Biology (Shanghai, China) and cultured in DEMN supplemented with 100 U/ml streptomycin and 10% heat-inactivated fetal bovine serum (Hyclone). HaCaT cells were kept at 37 °C and 5% CO_2 in humidified atmosphere.

2.2. siRNA transfection

The three pairs of siRNA (sigma) duplexes targeting STAT1 and STAT3 gene, and a pair of negative control siRNA (NC-siRNA) with no complementary target sequence were obtained commercially. HaCaT cells were seed into 6-well plates at a density of 5×10^4 cells/well approximately 48 h before transfection. When HaCaT cells were 40–50% confluent, 5 μL lipofectamine-2000 (Invitrogen) was added to 500 μL opti-MEM Serum Medium (GIBICO), mixed gently, and incubated for 5 min at room temperature. In parallel, 2.5 μL, 5 μL, 10 μL siRNA (20 μmol/L) were separately added to 500ul opti-MEM Serum Medium, mixed with the lipofectamine-2000. Then STAT1-siRNA, STAT3-siRNA and control siRNA with the final concentrations of 25 nmol/L, 50 nmol/L and 100 nmol/L were respectively introduced into HaCaT. The efficiency of gene knockdown was evaluated by Q-PCR and Western-blot after incubation in normal cell culture conditions for 24 h.

2.3. Acitretin effection

2.3.1. MTS assay of cell viability

HaCaT cell viability was assessed using 3-(4,5-di-methyl-thiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Briefly, HaCaT cells induced with the chosen siRNA-STAT1 and siRNA-STAT3 were seeded at their optimal cell density (1×10^5 cells/well) in 96-well plates. Meanwhile, eight groups containing cells group (A), lipofectamine-2000 group (B), NC-siRNA group (C), acitretin group (D), STAT1-siRNA group (E), STAT3-siRNA group (F), STAT1-siRNA + acitretin group (G), STAT3-siRNA + acitretin group (H) were set up. The cells were treated with 5 μmol/L acitretin (selleckchem) for 12, 24, 48 and 72 h respectively. At end of each incubation period, cells were mixed with 10ul MTS (Promega) and incubated for 4 h at 37 °C in CO_2 incubator. Cell viability was metered by measuring the optical density (OD) at 490 nm.

2.3.2. Q-PCR and Western blot analysis of acitretin affection on STAT1/3 and SOCS1/3

The selected STAT1-siRNA and STAT3-siRNA were transfected to HaCaT cells which were treated with or without 5 mol/L acitretin. The eight groups' expression of STAT 1/3, SOCS 1/3 mRNA and protein were determined by Q-PCR and western after incubation for 24 h. Detailed primer sequences for STAT1, STAT3, SOCS1, SOCS3 and GAPDH were given as follows: STAT1 forward 5′ATT ACA AAG TCA TGG CTG CT3′, reverse 5′ATA TCC AGT TCC TTT AGG GC3′; STAT3 forward 5′ CAT CTT GAG CAC TAA GCC T 3′, reverse 5′ GAG ATA GAC CAG TGG AGA CA 3′; SOCS1 forward 5′AGC TTC GAC TGC CTC TTC3′, reverse 5′GGA AGG AGC TCA GGT AGT C3′; SOCS3 forward 5′GAC GGA GAC TTC GAT TCG3′, reverse 5′AAA CTT GCT GTG GGT GAC3′; GAPDH forward 5′ TCC ACT GGC GTC TTC ACC ACC AT 3′, reverse 5′ GGA GGC ATT GCT GAT GAT CTT GAG G 3′. Relative expression of STAT1/3 and SOCS1/3 were normalized to GAPDH.

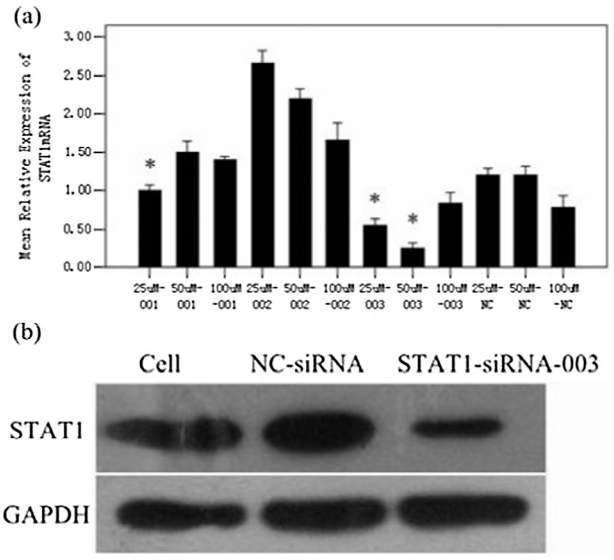

Fig. 1. Relative expression of STAT1. The level of STAT1mRNA in HaCaT cells were detected by Q-PCR (a). (*p < 0.05 vs NC-siRNA). The protein content of STAT1 in HaCaT cells which was transfected with 50 nMol/L STAT1-siRNA-003 (b).

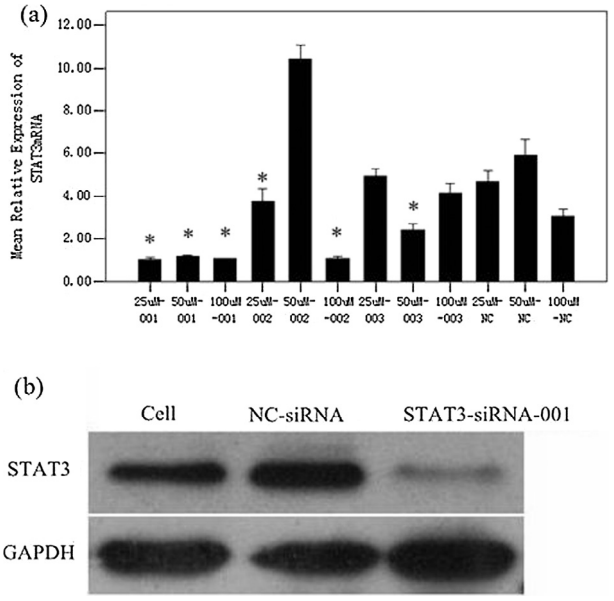

Fig. 2. Relative expression of STAT3. The level of STAT3mRNA in HaCaT cells were detected by Q-PCR (a). (*p < 0.05 vs NC-siRNA). The protein content of STAT3 in HaCaT cells which was transfected with 50 nMol/L STAT3-siRNA-001 (b).

2.4. Statistical analysis

Dates were expressed as mean ± SD for three independent experiments. Comparisons among groups were carried out using One-Way ANOVA. In all analyses, P value < 0.05 was deem to demonstrate statistical significance.

3. Results & discussion

Three pairs of STAT1-siRNA and STAT3-siRNA were independently introduced into HaCaT cells at 25, 50, 100 nmol/L. As shown in the Q-PCR analysis, compared with the control group, STAT1 and STAT3 mRNA were down-regulated by STAT1-siRNA and STAT3-siRNA, respectively (Fig. 1a, Fig. 2a). As for the protein levels, STAT1