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Original article

MicroRNA138 regulates keratin 17 protein expression to affect HaCaT cell proliferation and apoptosis by targeting *hTERT* in psoriasis vulgaris

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ABSTRACT

The purpose of this study is to explore the how microRNA-138 (miR-138) affects the expression of keratin 17 (K17) and psoriasis development. Twenty-eight skin lesions from patients with psoriasis vulgaris and twenty-four normal skin tissues from healthy controls were collected. The HaCaT cells were assigned into blank, negative control (NC), miR-138 mimic, miR-138 inhibitor, hTERT siRNA and miR-138 inhibitor + hTERT siRNA groups. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to detect the miR-138 expression. The hTERT and K17 protein expression were testified by Western Blotting. MTT assay, flow cytometry with PI single staining and Annexin V/PI double staining were performed to detect the cell proliferation activity, cell cycle and apoptosis, respectively. Compared with the healthy skin, the expression of miR-138 decreased in the psoriatic skin, but hTERT and K17 protein expressions increased. The miR-138 mimic and hTERT siRNA groups showed significantly decreased hTERT and K17 protein expressions, inhibited cell proliferation, increased number of cells at G1 phase and elevated apoptosis rate in comparison to the rest three groups. The hTERT and K17 protein expressions in the miR-138 inhibitor group were up-regulated with promoted cell proliferation and reduced apoptosis rate as compared with the other four groups. In the miR-138 inhibitor + hTERT siRNA group, the hTERT and K17 protein expressions, cell proliferation and apoptosis were intermediate between the miR-138 inhibitor and hTERT siRNA groups. These findings indicated that the expression of miR-138 was lower in the psoriatic skin, which was negatively correlated to K17 expression. MiR-138 may regulate K17 protein expression to affect HaCaT cell proliferation and apoptosis by targeting *hTERT* gene.

1. Introduction

Psoriasis is a T-cell mediated, chronic, inflammatory skin disease, of which psoriasis vulgaris is the most common type with major clinical symptoms of scaly debris and invasive erythema along with different degrees of itching [1]. The prevalence of psoriasis worldwide is about 2%–3% and it mostly occurs in adults over 39 years old [2]. Currently, the etiology and pathogenesis of psoriasis are not clear, but it has been reported that some factors, including genetic factors, environmental factors, immunological mechanisms, new blood vessel formation, lipid metabolism disorders and unhealthy mentality, bear significant impacts on the occurrence of psoriasis [3]. It has been reviewed that general treatments of psoriasis are topical treatments, ultraviolet radiation b, psoralen/ultraviolet-A radiation (PUVA), methotrexate, cyclosporin-a, acitretin and biological agent therapies [4]. As the exploration into the development and progression of psoriasis, new therapies concerning cytokines, signaling molecules, and genes such as microRNAs (miRs) target gene have become potential therapeutic targets for psoriasis vulgaris [5,6].

MiRs are banded to the 3′ untranslated regions (UTR) of mRNAs, which inhibits translation process and leads to accelerated turnover or degradation of the miRNA transcript [7]. Accordingly, abnormal expressions of miRs have been demonstrated to be associated with the occurrence of cancers [8,9], heart diseases [10], inflammatory diseases [11], and other medical conditions, which suggest that miRs can act as potential targets for medical diagnosis. The role of certain specific miRs in psoriasis has also been studied recently, such as hsa-miR-99a [5], miR-146a [12], and miR-125b

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[13]. As a member of miRs, miR-138 is involved in a variety of physical processes such as tumor metastasis and differentiation, DNA damage and cell senescence through regulating target genes [14,15]. Previous evidences have shown that the overexpression of miR-138 can induce a decrease in expression of human telomerase reverse transcriptase (hTERT) protein, which regulats telomerase activity in various cancers, such as malignant neuroblastoma, human anaplastic thyroid cancer and cervical cancer [11,16,17]. Interestingly, psoriasis is commonly characterized with the hyperproliferative state of keratinocytes and highly activated telomerase [18]. In addition, Keratin 17 (K17), compared to in normal epidermis, is reported to be abnormally expressed in the suprabasal keratinocytes of psoriatic lesions, [19]. K17 belongs to the group of human type I (acidic) epithelial keratins which help to keep the integrity of the epidermis by providing mechanical support to keratinocytes [20,21]. However, the functions of miR-138 and *hTERT* gene are still not well understood in the specific skin diseases, such as skin lesions of psoriasis vulgaris. In this regard, we performed this study to explore the effects of miR-138 expression on psoriasis development by targeting the *hTERT* gene and we investigated how miR-138 affects the expression of K17, which may be a potential target for diagnosis and therapy of psoriasis vulgaris.

2. Material and methods

2.1. Study subjects

We recruited 28 patients with psoriasis vulgaris (15 males and 13 females; average age: 31.4 ± 9.7 years old) who were admitted into the Department of Dermatology of Cangzhou Central Hospital between June 2013 and January 2015. Patients who met the following criterion were eligible for this study: 1) patients pathologically diagnosed as psoriasis vulgaris; 2) patients with no consumption of drugs, such as immunosuppressant, glucocorticoid or vitamin A acid within three months before sample collection; 3) patients with no external use of drugs or light therapy for psoriasis vulgaris within at least 1 month before sample collection; 4) patients without other types of skin diseases, other autoimmune diseases, tumors or other serious diseases. Patients in pregnancy, lactation or menstrual period were excluded. Additionally, a total of 24 patients receiving surgery at the Department of Burn were recruited as the control group, consisting of 11 males and 13 females with the mean age of 32.2 ± 10.1 years old. The inclusion criteria of the control group were: 1) no history of psoriasis and family history of disease; 2) no other immunological diseases; 3) no tumor or other severe diseases. Psoriatic skin and healthy skin were stored in liquid nitrogen. There was no significant difference of gender and age between the case and control groups (both $P > 0.05$). The experiment was approved by the Ethics Committee of Cangzhou Central Hospital and all subjects signed informed consent.

2.2. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA were extracted using miRNAeasy Mini Kit (Qiagen Company, Hilden, Germany). The concentration and purity of all RNA samples were detected by ultraviolet spectrophotometer at 260 nm and 280 nm. Optical density (OD) 260/OD280 ratio between 1.7–2.1 demonstrated a higher purity of RNA. Total RNA samples were reverse transcribed to synthesize complementary DNA (cDNA). qRT-PCR was performed on an ABI7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The PCR reaction conditions: Pre-degeneration at 95 °C for 10 min, denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s, extension at 72 °C for 34 s with a total of 40 cycles. MiR-138 upstream primer sequence: 5′-GGTGTCGTGGAGTCGGCAA-3′, downstream primer sequence: 5′-AACTTCACAACACCAGCTTA-3′; U6 upstream primer sequence: 5′-CTCGCTTCGGCAGCACA-3′, downstream primer sequence: 5′-AACGCTTCACGAATTTGCGT-3′. U6 gene was used as the internal reference. $2^{-\Delta\Delta Ct}$ referred to the ratio of targeted gene expression between the experiment and control groups, with formula as $\Delta\Delta CT = \Delta Ct_{\text{experiment\_group}} - \Delta Ct_{\text{control\_group}}$ ($\Delta Ct = Ct_{miRNA} - Ct_{U6}$). Ct referred to the cycle threshold that the real-time fluorescence intensity took to reach the expected threshold.

2.3. Western blotting

The skins of psoriasis patients and healthy individuals were collected and treated with neutral protease at 4 °C overnight. After complete grinding, cell protein lysate was added and samples were centrifuged at 2000 rpm for 2 min. Then, the supernatant was obtained, the concentration of which was examined using bicinchoninic acid (BCA) kit (Thermo Fisher Scientific, California, USA). The supernatant was boiled for 5–10 min and then stored at −20 °C. The extracted protein underwent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for 1 h and then was transferred to polyvinylidene fluoride (PVDF) membrane for 20 min and sealed in milk solution for 2 h. With the addition of rabbit anti-human K17 and mouse anti-human, the membrane was maintained overnight at 4 °C. Subsequently, HRP-labeled goat anti-rabbit IgG antibody and HRP-labeled rabbit anti-mouse IgG antibody were added and samples were incubated at 37 °C for 1 h. The primary and second antibodies were purchased from Santa Cruz Biotechnology, CA, USA. Chemiluminescent visualization was performed in dark condition according to the instruction of electrochemical luminescence (ECL) color kit. Inner reference was at GAPDH.

2.4. HaCaT cell culture

HaCaT cells (Wuhan Procell life Technology Co., Ltd., Hubei, China) were cultured in dulbecco's modified eagle medium (DMEM)/F12 (Gibco, Gaitherburg, MD, USA) containing 10% fetal calf (Hyclone, MA, USA) at 37° C in a humidified incubator with 5% CO_2. The nutrient solution was refreshed every 2 days. When cells reached 80% confluence, they were passaged and digested with 0.25% pancreatin for 8 min, supplemented with 10% serum-containing culture medium. When reaction was terminated, the cells were seeded into culture bottle.

2.5. Luciferase reporter gene assay

DNA extraction was conducted according to the instruction of TIAN amp Genomic DNA Kit (TIANGEN Biotech (Beijing) Co., Ltd., China). Luciferase reporter vector was constructed. The luciferase reporter vectors of wild type 3′UTR (Wt-3′-UTR) and mutant type 3′UTR (Mut-3′-UTR) of *hTERT* gene were co-transfected with miR-138 mimic or negative control, respectively, into the HaCaT cells. Luciferase activity of samples was detected using luciferase reporter assay system (E1910) (Promega Corporation, Madison, WI, USA). After 48 h transfection, the old medium was removed and sample was then washed by phosphate buffered saline (PBS) twice. With the addition of 100 μl passive lysis buffer (PLB) in each well, samples were slightly shaken at room temperature for 15 min, after which the cell lysates was collected. We set a program pre-reading at 2 s and program reading at 10 s, and set the injection volume of LARII Stop & Glo® Reagent at 100 μl each time. Then the LARII Stop & Glo® Reagent and luminotrons or plates with prepared cell lysates (20 μl of each sample) were put into the lumi-aggregometer.