Biomedicine & Pharmacotherapy 91 (2017) 803–811

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

MiR-330 inhibits IL-22-induced keratinocyte proliferation through targeting CTNNB1

Hui Shen[a,b], Bijun Zeng[a], Chang Wang[a], Xueyong Tang[a], Haizhen Wang[a], Wen Liu[a], Zhibo Yang[a,*]

[a] Department of Dermatology, The Second Affiliated Hospital of Hunan University of Chinese Medicine, Changsha, Hunan Province, 410005, China
[b] Department of Dermatology, Ruikang Hospital affiliated to Guanxi University of Chinese Medicine, Nanning, Guangxi Province, 530000, China

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form 25 April 2017
Accepted 2 May 2017

Keywords:
MiR-330
IL-22
CTNNB1
Keratinocyte
Psoriasis

ABSTRACT

Psoriasis is a common chronic inflammatory skin disease which is characterized by hyperproliferation and aberrant differentiation of keratinocytes; however the exact pathogenesis is largely unknown. Interleukin-22 (IL-22) has demonstrated its vital role in T cell-mediated immune response by interacting with keratinocytes in the pathogenesis of psoriasis. The microRNAs (miRNAs) are a class of small non-coding RNA molecules that play important roles in cellular processes by regulating gene expression at the post-transcriptional level. MiR-330 has been reported to inhibit the proliferation and migration of mouse keratinocytes. In the present study, we indicated that miR-330 expression in lesion tissue of psoriasis patients was specifically down-regulated, and could inhibit IL-22-induced proliferation of HaCaT and HKC cell. Wnt/β-catenin pathway plays an essential role in the pathogenesis of psoriasis. By direct targeting CTNNB1, miR-330 could significantly downregulate IL-22-induced CTNNB1 expression. In addition, we found that the downstream targets of β-catenin, CyclinD1 and Axin2, could be affected by miR-330; miR-330 could suppress CyclinD1 protein expression and rescue Axin2 protein expression. Taken together, we indicated miR-330 inhibits IL-22-induced proliferation of HaCaT and HKC cell by targeting CTNNB1 and subsequently affect the downstream factors, CyclinD1 and Axin2 for the first time, and provide diagnostic markers and a novel target for psoriasis treatment.

1. Introduction

Psoriasis is a chronic, immune-mediated inflammatory skin disease which is characterized by abnormal hyperplasia and differentiation in keratinocytes, resulting in histopathologic changes, including thickening of the epidermis and parakeratosis [1–3]. Even though the underlying mechanisms remain largely elusive, immunological dysfunction plays a crucial role in the process of this disease. In particular, immune response mediated by T cells and keratinocytes has participated in the initiation and maintenance of psoriasis [3]. Numerous inflammatory cytokines produced by immune cells contribute to the proliferation of keratinocytes [4]. Interleukin-22 (IL-22), belonging to the IL-10 superfamily [5], is mainly produced by activated Th1, Th17, Th22 and acts only on keratinocytes rather than immune cells in the skin [6–8]. The expression of IL-22 mRNA is massively increased in lesions and serum from psoriatic patients and it is correlated with the disease severity [9,10]. In addition, IL-22 promotes keratinocyte proliferation, induces keratinocyte migration and down-regulates the expression of genes associated with keratinocyte differentiation *in vitro* [11,12]. All evidence strongly suggests that IL-22 plays a critical role in the pathogenesis of psoriasis.

MicroRNAs (miRNAs) are small single-stranded noncoding RNAs that play important roles in the physiological and pathological processes in human beings. They can negatively regulate the expression of target genes at the post-transcriptional level by binding to the 3'untranslated region (UTR) of the target mRNA [13]. Recent studies have demonstrated that altered miRNA profiles were linked to the pathogenesis of psoriasis, thus opening up new horizons for the research on psoriasis [14–18].

The Wnt/β-catenin pathway is best known for its role in embryogenesis and development [19,20]. Some researchers highlight that the Wnt/β-catenin pathway is also involved in the regulation of cancer cell proliferation [21,22]. In addition,

* Corresponding author at: No.233 Caie North Road, Tianxin District, Changsha, Hunan Province, 410005, China.
E-mail address: dr_yangzhibo@163.com (Z. Yang).

http://dx.doi.org/10.1016/j.biopha.2017.05.005
0753-3322/

β-catenin has been reported to be promoted keratinocyte proliferation [23,24]. In the present study, we investigated the functional role of miR-330 in the regulation of IL-22 induced keratinocyte proliferation. Further, we investigated whether miR-330 exerts its functions through β-catenin signaling and the downstream target factors. Taken together, these findings provided a novel molecular basis for the potential effect of miR-330 in the treatment of psoriasis.

2. Materials and methods

2.1. Clinical specimens

With the approval of the Ethic Committee of The Second Affiliated Hospital of Hunan University of Chinese Medicine, we collected 26 paired psoriatic skin lesional tissues and normal non-lesional tissues from December 2014 to June 2016. 3 mm punch biopsies were taken from lesional skin. The non-lesional skin was taken about 5 cm away from the lesion biopsy. All psoriasis patients were diagnosed by dermatologists according to diagnostic standard for psoriasis. All cases signed informed consent. All the tissue samples were snap-frozen and stored at −80 °C in liquid nitrogen.

2.2. Isolation of primary human keratinocytes

Adult keratinocytes were obtained directly from discarded skin from abdominoplasties. Excess human skin from abdominoplasty surgery was harvested as 350–400 μm thickness skingrafts. Skin was washed in PBS three times, cut into pieces approximately 5 × 5 mm, and treated with 5 mM EDTA in PBS for 90–120 min, after which the epidermis was peeled from the dermis with forceps and minced with a scalpel. The epidermal mince was treated with 1 × trypsin–EDTA for periods up to 30 min on a magnetic stirrer at 37 °C. Treated cells were passed through a 70 μm strainer, before counting on a haemocytometer.

2.3. Cell culture and treatment with cytokine IL-22

Human keratinocyte HaCaT cells purchased from China Center for Type Culture Collection (Wuhan, China) and cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum with 5% CO_2 in a 37 °C incubator. The isolated human primary keratinocytes were cultured in EpiLife® basal medium (Gibco, USA) supplemented with 1 × Human Keratinocyte Growth Supplement (Gibco, USA), 100 units/mL penicillin and 100 μg/ml streptomycin (Beyotime, China) in a 37 °C incubator with 5% CO_2.

To prepare them for recombinant human IL-22 (Sangon Biological Engineering Technology Company, Shanghai, China) treatment, HaCaT cells and HKCs were starved in serum-free DMEM for 24 h and then treated with IL-22 (100 ng/ml) in serum-free DMEM for another 24 h or not treated.

2.4. Lentivirus production

The lentivirus vector of miR-330 and anti-miR-330 was obtained from the Shanghai R&S Biotechnology (Shanghai, China), and cotransfected with helper plasmids (pLP1-Gag-pol, pLP-VSVG, and pLP2-Rev) into human embryonic kidney 293T cells (China Center for Type Culture Collection, Wuhan, China). The supernatant was collected at 48 h after transfection, and fresh medium was added to the culture flask. After the cells were cultured for another 24 h, the supernatant was collected again. The supernatants collected from 48 and 72 h were mixed, and the mixture was then centrifuged at 3000 rpm for 15 min at 4 °C. The liquid was filtered by a 0.45-mm filter membrane, and the acquired virus was stored at −80 °C until use. The titers of the lentivirus were 3×10^8 transfer units per ml (TU/ml).

2.5. Lentivirus infection

The cells were placed into 10 cm dishes (5×10^5 cell/dish) one day before the lentivirus infection. The next day, when the confluence reached 70%, the lentivirus was added into the dishes, with a MOI (multiplicity of infection) of 15, to infect HaCaT cells and HKCs. The infection efficiency was detected by a fluorescence microscopy analysis of GFP 24 h after infection, and the efficiency was ensured higher than 90%.

2.6. RNA extraction and SYBR green quantitative PCR analysis

We extracted total RNA from cells and tissue samples using Trizol reagent (Invitrogen, CA, USA) and detected mature miR-330 expressions using a Hairpin-it TM miRNAs qPCR kit (Genepharma, Shanghai, China). We used expression of RNU6 B as an endogenous control. CTNNB1 expression was measured by SYBR green qPCR assay (Takara, Dalian, China). Data were processed using $2^{-\Delta\Delta CT}$ method.

2.7. MTT assay

A modified MTT assay was used to evaluate cell viability. 24 h after seeded into 96-well plates (5000 cells per well), cells were transfected with LV-miR-330 or LV-anti-miR-330 with the presence or absence of IL-22 treatment. 48 h after transfection, 20 μl MTT (at a concentration of 5 mg/ml; Sigma-Aldrich) was added, and the cells were incubated for an additional 4 h in a humidified incubator. 200 μl DMSO was added after the supernatant discarded to dissolve the formazan. OD_{490nm} value was measured. The viability of the non-treatment cells (control) was defined as 100%, and the viability of cells from all other groups was calculated separately from that of the control group.

2.8. BrdU incorporation assay

DNA synthesis in proliferating cells was determined by measuring 5-Bromo-2-deoxyUridine (BrdU) incorporation. BrdU assays were performed at 24 h and 48 h after transfecting HaCaT cells and HKCs with LV-miR-330 or LV-anti-miR-330. After seeding the infected cells in 96-well culture plates at a density of 2×10^3 cells/well, they were cultured for 24 h or 48 h, and incubated with a final concentration of 10 μM BrdU (BD Pharmingen, San Diego, CA, USA) for 2 h to 24 h. When the incubation period ended, we removed the medium, fixed the cells for 30 min at RT, incubated them with peroxidase-coupled anti-BrdU-antibody (Sigma-Aldrich) for 60 min at RT, washed them three times with PBS, incubated the cells with peroxidase substrate (tetramethylbenzidine) for 30 min, and measured the absorbance values at 450 nm. Background BrdU immunofluorescence was determined in cells not exposed to BrdU but stained with the BrdU antibody.

2.9. Western blot analysis

The expression of CTNNB1, CyclinD1 and Axin2 in lung cancer cells was detected by performing immunoblotting. We lysed cultured or transfected cells in RIPA buffer with 1% PMSF, and loaded protein onto a SDS-PAGE minigel and transferred them onto PVDF membrane. After probed with the following antibodies: CTNNB1 (rabbit monoclonal, Cat# E247, Abcam, MA, USA),