Journal of Dermatological Science 68 (2012) 157–163

Contents lists available at SciVerse ScienceDirect

Journal of Dermatological Science

journal homepage: www.elsevier.com/jds

Versican gene: Regulation by the β-catenin signaling pathway plays a significant role in dermal papilla cell aggregative growth☆

Yadong Yang [a,1], Yuanchao Li [b,1], Yuanyuan Wang [a], Jinjin Wu [c,*], Guihong Yang [a], Tao Yang [a], Yang Gao [a], Yuangang Lu [a]

[a] Department of Plastic Surgery, Daping Hospital, Research Institute of Surgery, Third Military Medical University, Chongqing 400042, PR China
[b] Department of Dermatology, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, PR China
[c] Department of Dermatology, Daping Hospital, Research Institute of Surgery, Third Military Medical University, Chongqing 400042, PR China

ARTICLE INFO

Article history:
Received 4 February 2012
Received in revised form 3 September 2012
Accepted 14 September 2012

Keywords:
Dermal papilla cells
Versican
Wnt/β-catenin

ABSTRACT

Background: Dermal papilla cells (DPCs), which exhibit a multilayer aggregative growth character in *in vitro* culture, are closely related to induction of hair follicles (HFs) formation, and are associated with the development and cycle regulation of HFs. Versican, a large chondroitin sulfate proteoglycan and one of the major components of the extracellular matrix, plays an essential role in hair follicle formation. And also the Wnt/β-catenin signaling pathway performs a crutial function in induction during hair follicle growth and embryogenesis.
Objective: To characterize the role of versican and β-catenin in regulating DPCs aggregative growth, and to explore the versican gene expression regulation mechanism by TCF-4/β-catenin signaling pathway.
Methods: We first cultured DPCs at different passages, and detected the change in β-catenin and versican expression in DPCs of various passages by RT-PCR and Western blot. Then we knockdowned the versican and β-catenin gene, evaluated and verificated the binding capability of TCF-4/β-catenin to TOP elements in versican gene promoter region at varied passage DPCs by EMSA and ChIP Assay, finally observed the effect of Wnt/β-catenin pathway inhibition on DPC aggregative growth.
Results: With the increase of passage, DPCs lost the aggregative property, the versican mRNA and protein level in DPCs was on a gradual decline, while not significant declining tendency of β-catenin. The mRNA of both β-catenin and versican reduced simultaneously after β-catenin siRNA transfection. The binding ability of TCF-4/β-catenin of varied-passage DPCs to cultured versican promoters diminished with the increase of DPC passages. And versican inhibition or Wnt/β-catenin pathway blocking could both produce considerable effect on the aggregative growth of low-passage DPCs.
Conclusion: Wnt/β-catenin signal transducting system regulates DPC aggregative growth through modifying versican expression by means of acting on the versican gene upstream promoter.

1. Introduction

Dermal papilla cells (DPCs) have been proposed to serve as the basis of hair follicle formation and to be closely connected with hair follicle differentiation and functions [1,2], due to their aggregative growth property in culture *in vitro*. Meanwhile, the formation and growth of miniorgans are closely linked to extracellular matrix. In developmental biology, the extracellular matrix has drawn increasingly wide attention [3–6]. Mainly present in the extracellular matrix, versican protein, a chondroitin sulfate proteoglycan with a large molecular mass, plays a significant role in cell adhesion, migration, proliferation, and differentiation, thus it is also a multifunctional proteoglycan [7]. Previous researche has indicated that the protein expression of versican reaches a maximum during anagen, declines at the end of anagen, further decreases during catagen, and vanishes from the structure during telogen [8]. Also some research has confirmed that versican disappears rapidly from culture during the passaging of DPCs. Non-versican-expressing DP cells however, lose their hair follicle-regeneration-supporting ability [9]. These findings suggest versican expression plays an essential role in hair follicle formation

☆ This work was supported by grants from NCSF (No. 30771934) and CSTC (No. 2009BB5161).
* Corresponding author at: Department of Dermatology, Daping Hospital, Research Institute of Surgery, Third Military Medical University, No. 10 Changjiang branch Rd., Daping Yuzhong District, Chongqing 400042, PR China.
Tel.: +86 23 68757591; fax: +86 23 68757591.
E-mail address: wjjjj@163.com (J. Wu).
[1] These authors contributed equally to this work.

0923-1811/$36.00 © 2012 Japanese Society for Investigative Dermatology. Published by Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jdermsci.2012.09.011

and its function is dose-dependent to some degree. Another signaling pathway, Wnt/β-catenin, performs a crucial function in induction during hair follicle growth and embryogenesis [10]. As a basic signaling molecule in follicle substrate formation, it guides the direction of hair follicle stem cell differentiation after hair follicle formation [9]. As is known to us, when the Wnt signals are inhibited, skin substrate cannot be formed; the embryonic bud is not produced; and the development of hair follicles, teeth and mammary glands is also interrupted [10].

This study aimed to characterize the key role of Wnt/β-catenin signaling pathway in regulating DPC aggregative growth through comparing the aggregative growth status and intracellular expression of β-catenin and versican in DPCs of various passages.

2. Materials and methods

2.1. Observation of the aggregative growth property in DPCs at different passages

DP cells were isolated from occipital haired region of the scalp from a healthy adult male, subject to the approval of the Review Board of Daping Hospital of Third Military Medical University, with the "Two-step Enzyme Digestion" method. After the removal of hair from the fresh, full-thickness scalp, the fascia tissues were isolated with great caution along the interface between subcutaneous fatty tissues and fascia. Next, the cleaned samples were cut into strips (width: 0.3–0.5 cm; length: 3–4 cm), which were then separated along the interface between the dermis and subcutaneous fat layer. Dermal tissues were discarded and the subcutaneous fat was retained and immersed with 0.25% dispase and digested at 4 °C overnight (about 12–16 h). After that, the hairs were extruded gently along the hair follicle direction with nippers. The fatty tissues were cut to the size of a grain of rice, incubated with 0.2% type IV collagenase and digested at 37 °C for 4–6 h, followed by gradient centrifugation (2000 rpm, 1000 rpm, 500 rpm, 5 min/per time × 4 times). Subsequent to the removal of the supernatant, DMEM medium containing 10% fetal calf serum was added and the fresh cells were inoculated for culture. After the majority of the hair papilla cells had adhered, the medium was changed and the non-adhered impurities were eliminated. Every 3–5 days, the culture medium was changed. The cells were serially subcultivated after reaching the fusion state. The growth of the 1st–7th passage DPCs cultured *in vitro* was regularly observed under the microscope.

2.2. Change in β-catenin and versican expression in DPCs at different passages

The mRNA level of β-catenin and versican in DP cells during the 1st–7th passages cultured *in vitro* was determined by PCR fluorescent quantitation. DP cells of various passages were collected, and 10^6 cells from each passage were incubated with 1 mL Trizol reagent for RNA extraction. Total RNA (2 μg) was reverse-transcribed into cDNA with the PrimeScript™ 1st Strand cDNA Synthesis Kit (TAKARA). β-Actin was used as the internal reference for the real-time fluorescent quantitation PCR (SYBR Green method), and the relative gene expression of both β-catenin and versican was quantified by using their ratios to β-actin. The data were derived from 3 independent experiments.

The protein expression of β-catenin and versican in DP cells during the 1st–7th passages was evaluated with a Western blot assay. After cell lysis and centrifugation (13000 rpm × 10 min), 150 μl of supernatant was added with 50 μl of 4× loading buffer and placed in boiling water. The SDS gel was electrophoresed at 120 V, transferred to PVDF membrane, and the membrane was incubated with mouse anti-human β-catenin and rabbit anti-human versican antibody (diluted at 1:1000). After 3 washes with TBS-T, addition of secondary antibody, and another TBS-T wash, the membrane was scanned by an image scanning system, saved in electronic format, and semiquantitatively analyzed with the software Quantity One (Bio-Rad).

2.3. Effect of versican knockdown on the expression of β-catenin and versican in low-passage DPCs

The interaction between β-catenin and versican was observed after the knockdown of β-catenin and versican genes in the 2nd passage DP cells with siRNA (Sigma–Aldrich Corp.). Later, siPORT™ Amine (Ambion, USA) was transfected into the 2nd-passage DPCs to measure the RNA expression of β-catenin and versican genes in different periods with fluorescent quantitation PCR. The changes in protein expression of β-catenin and versican after transfection were assessed by Western blot. The real-time fluorescent quantitation PCR and Western blot assay, as well as statistical methods were performed as previously above.

2.4. Binding capability of TCF-4/β-catenin to TOP elements in versican gene promoter region

The principle means for β-catenin to regulate downstream genes is to form the TCF-4/β-catenin complex. For this reason, we evaluated the binding capability of TCF-4/β-catenin in DPCs at various passages to TOP elements in the versican gene promoter region by EMSA assay. The oligonucleotide probe with the same sequence as the versican promoter region was synthesized by Sangon Co., Ltd. (Shanghai), labeled by biotin at the 3′ end and annealed to generate double strands. The sequence of this oligonucleotide probe was 5′-ACTTCCCTTTGATGGGACAG-3′ and 5′-CTGTCCCATCAAAGGGAAGT-3′, which contained the reported TCF-4/β-catenin binding site TOP. The purified probe, together with the DPC nucleoprotein at different passages, was added to the DNA binding buffer (15 μl) and supplemented with deionized water to 19 μl. Following incubation at 37 °C for 30 min, loading buffer (4 μl) was added and mixed along with 1 μl of non-labeled AP21 probe (100 ng) and 1 μl of labeled probe (1 ng) which were used for the cold probe competition reaction. The reaction without nucleoprotein extract was used as the control. After constant-current electrophoresis (10–12 V/cm, 1–2 h) and autoradiography, optical density values (OD value) of the bands were compared. In addition, 24 h after the treatment of the 2nd-passage DPCs with Wnt/β-catenin pathway specific inhibitor SB-415286 (Sigma–Aldrich Corp.), the binding capability of TCF-4/β-catenin to the versican gene promoter region was assessed by EMSA Assay.

2.5. Further verification of the binding ability of TCF-4/β-catenin to the versican gene upstream promoter region by ChIP Assay

To further confirm the transcriptional control ability of β-catenin with respect to the versican gene, the binding ability of TCF-4/β-catenin to the putative TOP site in the versican gene upstream promoter region was determined by ChIP assay kit (Millipore). Firstly DP cells of various passages were fixed with 1% formaldehyde for 30 min, then collected and lysed by lysis buffer for 30 min, ultrasonicated at an output power of 20 W for 3 times (10 s each time) to break the genomic DNA into fragments of 200–500 bp. Later 1 μg of β-catenin antibody was added, and incubated overnight with shaking at 4 °C. Agarose-coupled Protein A containing salmon sperm DNA (60 μl) was added and incubated for another 2 h at 4 °C. After Protein A was washed 3 times, the specific protein-DNA complex was eluted, and the DNA was extracted with the phenol chloroform method.