Molecular and Cellular Endocrinology 343 (2011) 25–31

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Expression of proliferin-related protein in testis and the biological significance in testosterone production

Lina Zhao [a], Jie Hao [b], Jiangang Hu [c], Qi Wang [a], Zhilan Lü [a], Limin Wang [a], Qiubo Yu [a], Yingxiong Wang [a], Gang Li [a,*]

[a] *School of Public Health, Chongqing Medical University, Chongqing, China*
[b] *The First Affiliated Hospital of Chongqing Medical University, Chongqing, China*
[c] *Department of Neurosurgery, The People's Hospital of Dazu Country, Chongqing, China*

ARTICLE INFO

Article history:
Received 4 January 2011
Received in revised form 29 April 2011
Accepted 31 May 2011
Available online 6 June 2011

Keywords:
Proliferin-related protein
Leydig cell
Testosterone

ABSTRACT

Proliferin-related protein (PRP) was originally identified as an angiogenesis inhibitor in mouse placentas. Indeed, the tissue expression of PRP has mainly been documented in placentas. We report herein for the first time that PRP is expressed in male rat testes. Immunocytochemical and *in situ* hybridization results showed positive PRP immunostaining in Leydig cells. Immunofluorescent staining of PRP in the TM3 Leydig cell line indicates that PRP is located within the cytoplasm. The expression pattern of PRP in rat testis exhibited an age-related increase. HCG significantly up-regulated the level of expression of PRP in TM3 cells via the PKA pathway. To elucidate the function of PRP, experiments were conducted to examine the consequences of lentiviral-mediated RNA interference (RNAi) of PRP on testosterone production and expression of several genes involved in steroidogenesis. PRP silencing caused a decrease in HCG-stimulated testosterone production. In addition, PRP silencing attenuated the increase in PRLR mRNA following HCG stimulation. Moreover, the enhanced effect of PRL on HCG-induced testosterone production was also weakened following PRP silencing, indicating that PRP may be involved in PRL function through an effect on PRL receptor expression in response to stimuli. Taken together, these data suggest that PRP is regulated by HCG and plays roles in male reproduction, such as testosterone production.
© 2011 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The placenta of many rodents and primates is a source of hormones from the prolactin (PRL) family. In rats and mice, the number of placental hormones related to PRL is quite large, and includes placental lactogen, PRL-like proteins, PRL-related proteins, proliferin, and proliferin-related protein. These hormones have important functions during pregnancy (Alam et al., 2006; Bachelot and Binart, 2007; Haig, 2008; Soares et al., 2007; Wiemers et al., 2007).

Proliferin-related protein (PRP), a potent placental anti-angiogenic hormone related to PRL, was so named because of its close relationship to another PRL-related hormone (proliferin [PLF]; Bengtson and Linzer, 2000; Jackson et al., 1994; Linzer and Nathans, 1985). PLF is expressed at high levels in early gestation to mid-gestation (Lee et al., 1988). Conversely, from mid-gestation to late gestation, PRP is expressed at a high level (Colosi et al., 1988; Toft and Linzer, 2000).

PLF may be important for attracting maternal endothelial cells toward trophoblasts; a reduction in placental production of PLF is in keeping with a decrease in decidual neovascularization (Ma et al., 1997). PRP appears to be responsible for slowing angiogenesis in response to PLF and other angiogenic factors, preventing endothelial cells from resealing open vessels, and generating a barrier zone to limit the growth of maternal and fetal vessels across the placenta (Linzer and Fisher, 1999).

Expression of PRP was originally identified in mouse placentas (Linzer and Nathans, 1985). Until now, PRP expression was considered to be restricted to placental tissues. However, in our previous study in which we determined the effect of ginsenosides on rat spermatogenesis, we occasionally found a positive PRP signal in microarray hybridization of rat testis mRNA. Here we present the morphologic evidence that PRP is expressed in the testis and located in Leydig cells. HCG can up-regulate the expression of PRP *in vitro*. We have also analyzed the effect of PRP on testosterone production in TM3 cells. The results suggest that PRP maybe play a role in the male reproductive system.

Abbreviations: 3β-HSD 1, 3β-hydroxysteroid dehydrogenase 1; DIG, digoxigenin; LHR, LH receptor; PRL, prolactin; PRLR, PRL receptor; PLF, proliferin; PRP, proliferin-related rotein; StAR, steroidogenic acute regulatory.

* Corresponding author. Address: School of Public Health, Chongqing Medical University, Yixueyuan Road 1, Chongqing 400016, China. Tel.: +86 023 68485778; fax: +86 023 68485008.
E-mail address: lg168sn@yahoo.com (G. Li).

0303-7207/$ - see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2011.05.046

2. Materials and methods

2.1. Materials

DMEM/F-12 and fetal bovine serum (FBS) were purchased from HyClone (Logan, UT, USA). A protein extraction kit, BeyoECL Plus Western blotting detection reagent, and an annexin V-FITC apoptosis detection kit were purchased from Beyotime Biotechnology (Jiangsu, China). The BLOCK-iT™ HiPerform™ Lentiviral Pol II miR RNAi Expression System with EmGFP, lipofectamine 2000, and Trizol reagent were purchased from Invitrogen (Carlsbad, CA, USA). A DIG RNA Labeling Kit (SP6/T7) was purchased from Roche (Mannheim, Germany). PRP antibodies (rat monoclonal IgG_{2a}, sc-80531, and rabbit polyclonal IgG, sc-98474), Calphostin C, and H-89 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Testosterone immunoassay kits were obtained from HORA Biotech (Shanghai, China). A SYBR® PrimeScript® RT-PCR Kit (Perfect Real Time) was purchased from TaKaRa Biotech (Liaoning, China). PRL and HCG were purchased from ProSpec-Tany TechnoGene (Rehovot, Israel). All of the other chemicals were purchased from Sangon Biotech (Shanghai, China).

2.2. Animal experiments

Twenty-four male Sprague–Dawley rats at 4, 12, and 22 months of age were obtained from the Chongqing Medical University Animal Center. The animals were divided into three groups according to age, as follows: young group (4 months old, $n = 8$); middle-aged group (12 months old, $n = 8$); and old group (22 months old, $n = 8$). All of the rats were kept under 12:12-h light–dark environmental conditions throughout the course of the experiments. Rats were killed to obtain testes for an *in situ* hybridization assay, immunohistochemistry assay, and protein and RNA isolation. The animal protocols conformed to the protocols approved by the Chongqing Medical University Animal Care and Use Committee.

2.3. Cell culture

TM3 Leydig cells were cultured in F12-DMEM medium supplemented with 10% FBS. 293FT cells were cultured in DMEM containing 10% FBS. All of the cells were placed in an incubator containing 95% air and 5% CO_2 at 37 °C, and the media were replaced every 2 days.

2.4. Preparation of lentivirus targeting PRP and transfection of TM3 cells

To prepare lentivirus targeting PRP of TM3 Leydig cells, we adopted the BLOCK-iT™ HiPerform™ Lentiviral Pol II miR RNAi Expression System with EmGFP, which efficiently delivers the miR RNAi of the gene of interest to mammalian cells *in vitro* or *in vivo*. In our previous experiments, we have constructed three PRP-pcDNA™6.2-GW/EmGFP miR vectors. The three vectors reduced RPR mRNA levels to 35.5%, 47.9%, and 80.6%. The first resulting vector was selected for producing lentiviruses. The sequence of oligonucleotides encoding the miRNA for PRP RNAi was as follows: top, 5′-TGCTGAAACAAGTCCTTCAGATCACTGTTTTGGCCACTGACTGACAGTGATCTAGGACTTGTTT-3′; bottom, 5′-CCTGAAACAAGTCCTAGATCACTGTCAGTCAGTGGCCAAAACAGTGATCTGAAGGACTTGTTTC-3′.

Subsequently, the lentiviruses were produced in 293FT cells and the blasticidin-resistant colonies were screened according to the manufacturer's procedure.

2.5. Probe synthesis and in situ hybridization

Probe synthesis was performed using a DIG RNA Labeling Kit (SP6/T7), according to the instructions from the manufacturer. Briefly, a 338-bp cDNA fragment of rat PRP was generated by RT-PCR from rat whole testis and cloned into the pSPT18 vector. The resulting construct was linearized and served as a DNA template to synthesize DIG-labeled probes using T7 RNA polymerase for the antisense probe and SP6 RNA polymerase for the sense probe.

To identify the cell type expressing PRP mRNA, *in situ* hybridization was performed on 5-μm sections. Tissues were incubated in DEPC-treated PBS and fixed in 4% paraformaldehyde at 4 °C for 20 min. After rinsing twice with PBS, the slides were acetylated in 0.1 M triethanolamine–HCl buffer (pH 8.0) with 0.25% acetic anhydride for 10 min. The slides were washed in DEPC-PBS, rinsed with DEPC-H_2O, and pre-hybridized for 3 h at 50 °C. The DIG-labeled probe was diluted in hybridization buffer to 20 ng/μl and applied to the pre-hybridized tissues. The sections were then incubated at 80 °C for 2 min and hybridized at 48 °C for 18 h. Immunologic detection using anti-DIG-alkaline phosphatase with BCIP/NBT as substrates was performed according to the manufacturer's instructions. AP-conjugated anti-DIG antibodies (diluted 1:100 in PBS) were incubated with slides for 2 h. Finally, the slides were observed with an Olympus microscope (Tokyo, Japan).

2.6. Immunofluorescent staining of PRP

For immunofluorescent staining of PRP in TM3 cells, the TM3 cells were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature, followed by permeabilization through incubation in PBS containing 0.5% Triton X-100 at room temperature for 10 min. The cells were then immersed in blocking solution for 1 h. The cells were immunolabeled with rat anti-PRP primary antibody (diluted 1:100 in PBS) overnight at 4 °C. After three washes with PBS, the cells were incubated with fluorescein isothiocyanate-conjugated anti-rat IgG (1:200) at room temperature for 1 h at room temperature. The slides were finally analyzed by observed under a fluorescence microscope.

To verify whether or not PRP is expressed in Leydig cells, cellular co-localization of PRP with 3β-HSD1 was examined in the testes of adult rats using a double immunofluorescence staining method. Briefly, the sections were incubated for 48 h at 4 °C with a mixture of rabbit anti-PRP IgG and goat anti-3β-HSD1 IgG. Subsequently, the sections were rinsed in 0.01 M PBS (pH 7.4), then incubated for 24 h at 4 °C with a mixture of TRITC-conjugated anti-rabbit IgG and FITC-conjugated anti-goat IgG, and examined under an Olympus microscope.

2.7. Testosterone measurement

To analyze the effect of PRP silenced on testosterone production in TM3 cells, the TM3-NC cells (transfected with negative miR RNAi lentiviruses) and TM3-PRP-RNAi cells (transfected with PRP miR RNAi lentiviruses) were incubated for 6 h with or without HCG at 1 IU/ml, then the culture supernatants were collected for testosterone analysis. The limits of detection for testosterone ranged from 0.8 to 45.8 ng/ml, and the intra-assay and inter-assay CVs were 4.7% and 7.6%, respectively.

2.8. Effects of PRL on HCG-induced testosterone production in TM3-NC and TM3-PRP-RNAi cells

The TM3-NC and TM3-PRP-RNAi cells were cultured with 10 μg/l of PRL for 24 h followed by a 6-h restimulation of HCG, then the media were collected for testosterone detection.

Download English Version:

https://daneshyari.com/en/article/8477768

Download Persian Version:

https://daneshyari.com/article/8477768

Daneshyari.com