Gene 543 (2014) 212–219

Contents lists available at ScienceDirect

Gene

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

PLIN1 deficiency affects testicular gene expression at the meiotic stage in the first wave of spermatogenesis

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ARTICLE INFO

Article history:
Received 16 November 2013
Received in revised form 7 April 2014
Accepted 10 April 2014
Available online 13 April 2014

Keywords:
PLIN1
Lipolysis
Testis
Spermatogenesis
Reproduction

ABSTRACT

PLIN1, a lipid droplet associated protein, has been implicated in playing a key role in the regulation of lipolysis and lipid storage in adipocytes. *PLIN1* is found to be highly expressed in Leydig cells of testis, suggesting a potential role in steroidogenesis and spermatogenesis. In this study, we showed that *PLIN1* was expressed in testis and that its mRNA levels declined significantly with development. To investigate the role of *PLIN1*, we take advantage of *PLIN1*-null mice. We found that the number of seminiferous tubules containing round spermatids was significantly increased at P21 (postnatal day 21). Furthermore, microarray analysis showed that there were 538 differentially expressed genes between *PLIN1*-null and wild-type mice at P21. The up-regulated genes in knockout mice were enriched in spermatogenesis by Gene Ontology classification. Among them, *Prm1* and *Wbp2nl* are important for spermatogenesis which were confirmed by real-time PCR. Unexpectedly, the levels of serum testosterone and serum 17β-estradiol as well as steroidogenic genes are not altered in the *PLIN1*-null mice. Compared to the wild-type mice, no significant difference of fertility was found in the *PLIN1*-null mice. Therefore, these findings indicated that *PLIN1* disruption leads to the increase of round spermatid-containing seminiferous tubules at the meiotic stage of the first wave of spermatogenesis through regulating spermatogenic related genes.

1. Introduction

The stored lipids in lipid droplets (LDs) are precursors of steroid hormones, source of energy, and component of biological membranes (Farese and Walther, 2009). LDs are primarily filled with triacylglycerol in adipose tissue, while in adrenal cortex LDs store large amounts of sterol esters (Walther and Farese, 2012). It has been reported that the lipids of whole testis contain phospholipids, triacylglycerols, free and esterified cholesterol, gangliosides, and sulfolipids. The lipid compositions in various testicular cell types are different from each other. For example, late spermatids have the highest level of triacylglycerol in spermatids and spermatocytes (Coniglio, 1994). Lipids are important for the sperm cells, as lipids not only produce energy but also constitute the sperm plasma membrane (Lenzi et al., 1996). Lipid homeostasis also plays a critical role in the sex-hormones producing Leydig cells (Ge, 2007; Svechnikov et al., 2010). However, the role of lipids in spermatogenesis and steroidogenesis is still not fully understood.

LDs are targeted by several proteins, including PLIN1, which is involved in the dynamic regulation of lipolysis in LDs (Brasaemle et al., 2000). PLIN1 is a member of PAT protein family, encoded by the *PLIN1* gene (Bi et al., 2012; Kimmel et al., 2010). In adipocytes, PLIN1 protects lipids from cytosolic lipase and promotes lipid storage under basal conditions. PLIN1 is phosphorylated by protein kinase A (PKA) to facilitate lipolysis under energy deficient conditions (Brasaemle, 2007). The cholesterol ester-rich lipid storage droplets in steroidogenic cells are coated with PLIN1 (Brasaemle et al., 1997; Hsieh et al., 2012), similar to that in adipocytes.

The adipose tissue mass was reduced and the lipolysis in adipose tissue was significantly enhanced in *PLIN1*-null mice, due to constitutively activated hormone sensitive lipase (HSL) (Martinez-Botas et al., 2000; Tansey et al., 2001). A number of sterol biosynthetic enzymes were down-regulated in the adipose tissue of *PLIN1*-null mice (Castro-Chavez et al., 2003), implying that *PLIN1* deficiency may affect the sterol biosynthetic pathway in steroidogenic cells.

Here, we showed that *PLIN1* deficiency leads to an increase in the percentage of seminiferous tubules containing round spermatids by enhancing expression of meiotic genes. These data suggested that *PLIN1*

Abbreviations: P21, postnatal day 21; PAS, periodic acid-Schiff; Prm1, Protamine 1; Tnp2, transition protein 2; Wbp2nl, WW Domain-Binding Protein 2-Like; Fscn3, fascin homolog 3; LDs, lipid droplets; PKA, protein kinase A; HSL, hormone-sensitive lipase; BSA, albumin from bovine serum; CASA, computer-aided sperm analysis; PBS, phosphate-buffered saline; RT-PCR, reverse transcription polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SD, standard deviation; SEM, standard error; H&E, hematoxylin and eosin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Ecscr, endothelial cell surface expressed chemotaxis and apoptosis regulator; PTP1B, protein-tyrosine phosphatase 1B; IP6K1, inositol hexakisphosphate kinase 1.

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http://dx.doi.org/10.1016/j.gene.2014.04.021
0378-1119/

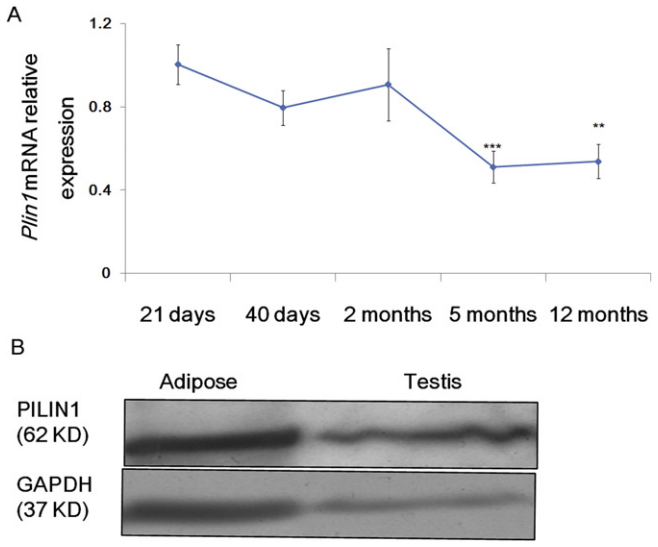

Fig. 1. Expression of *PLIN1* mRNA and protein in mouse testis. (A): Real-time PCR analysis of *PLIN1* mRNA during development. Results were obtained using $2^{-\Delta\Delta C_T}$ method and data were normalized to *GAPDH* mRNA. Data indicate mean ± SD (n = 3–5), **, $p < 0.01$; ***, $p < 0.001$. (B): Western blot showing PLIN1 protein in adipose and testis. GAPDH was used as the loading control.

plays an important role at early meiotic stage of the first wave of spermatogenesis.

2. Materials and methods

2.1. Ethics statement

All animal care and experimental procedures were approved by the Animal Ethical Committee of the State Key Laboratory for Agrobiotechnology (approval number: SKLAB-2010-09-01) and carried out according to its guidelines.

2.2. Animal studies

PLIN1-null mice in the 129/SvxC57BL/6 background were provided by Londos laboratory at the U.S. National Institutes of Health (Bethesda, MD, USA) and Guoheng Xu laboratory at Health Center of Peking University. Wild-type ($PLIN1^{+/+}$) and *PLIN1* homozygous knockout mice ($PLIN1^{-/-}$) were raised under conditions of regulated temperature (22 ± 2 °C), humidity (40 to 50%), and in 12 h light/12 h dark illumination cycles. Mice were provided with a standard rodent chow diet (1022 rat/mouse Maintenance Diet, HFK Bio-Technology Co. Ltd, CN) and water ad libitum.

Mice were fasted for 2 h before sacrifice. Blood samples were centrifuged at 1000 rpm for 20 min at 4 °C, and sera were collected for bioassays. Subsequently, testis, epididymis and epididymal adipose were collected.

2.3. Microarray analysis

Total RNA was extracted from testes of *PLIN1*-null mice and wild-type controls at P21, P40 and P60 using TRIzol (CWBIO, Beijing. P.R. China). Three *PLIN1*-null mice and three wild-type controls at each stage were used for microarray analysis. Preparation of cDNA targets, fragmentation, hybridization to Agilent Whole Mouse Genome Oligo Microarray (4 × 44K), washing, staining and scanning were performed following manufacturer protocols at Shanghai Biotechnology Co. Ltd. The microarray data was quantile normalized and linear modeled with the R package, Limma (Ritchie et al., 2007; Smyth, 2004, 2005). The microarray data can be accessed at GEO repository (GSE39769).

2.4. RT-PCR

Total RNA was extracted from testes using TRIzol Reagent (CWBIO, Beijing. P.R. China). First-strand cDNA was synthesized using the Superscript First-Strand kit (Promega) according to the manufacturer's instructions. Exon-spanning primers used for RT-PCR and Real time PCR are listed in Table S1. *GAPDH* mRNA was used as an internal control and results were calculated using $2^{-\Delta\Delta C_T}$ (Arya et al., 2005).

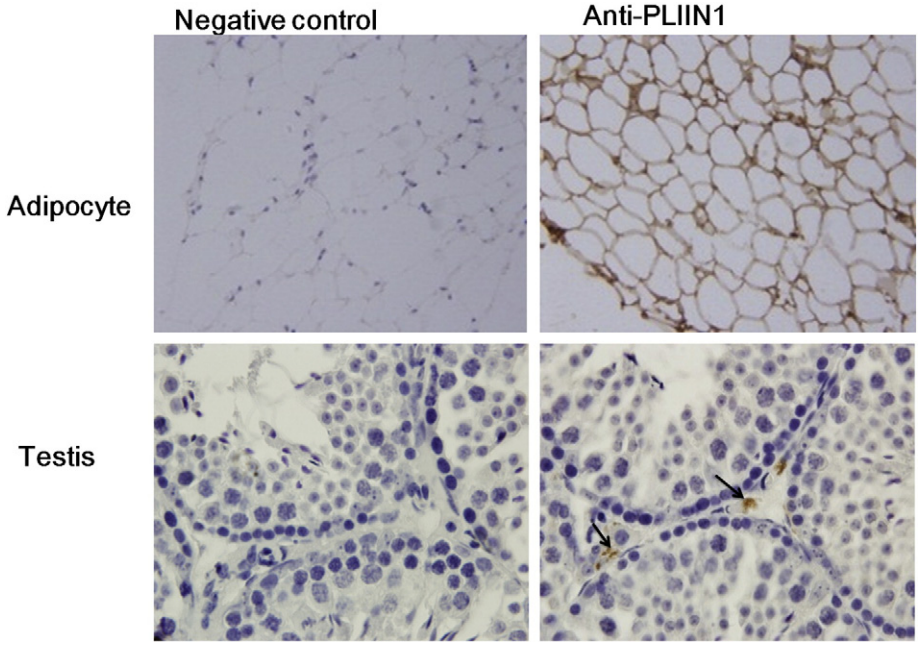

Fig. 2. Immunochemistry showing PLIN1 expression in adipocytes and Leydig cells at 40 days. PBS was used as the negative control. Magnification 100×.