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Prohibitin-1 is an ACTH-Regulated Protein in Human and Mouse Adrenocortical Cells and Plays a Role in Corticosteroid Production

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Abstract

Cell-intrinsic early events involved in different trophic hormone-induced steroidogenesis in their respective steroidogenic cell type are very similar. For example, the activation of the cAMP-PKA signaling pathway in response to trophic hormone stimulation and, subsequently, cholesterol transport to the mitochondria to initiate steroidogenesis is common to them. Recently, we have found that an evolutionarily conserved protein, prohibitin-1 (PHB1), is regulated by Luteinizing Hormone (LH) in murine Leydig cells and plays a role in interconnected cell signaling and mitochondrial steps pertaining to testosterone production. Among the primary steroidogenic tissues, PHB1 expression levels are highest in the adrenal cortex (The Human Protein Atlas); however, its regulation and role in adrenocortical cells are virtually unknown. We investigated the regulation and the role of PHB1 in adrenocortical cells in vitro using human HAC15 and mouse Y-1 cell culture models. It was found that Adrenocorticotrophic Hormone (ACTH) stimulation upregulates PHB1 levels in adrenocortical cells in a time-dependent manner. A similar effect on PHB1 levels was also observed in response to dibutyryl-cAMP stimulation, a cell-permeable analogue of cAMP (the second messenger for ACTH action). Moreover, manipulating PHB1 levels in adrenocortical cells affected mitochondria, lysosomes, and lipid droplet characteristics, modulated phospho-PKA and phospho-ERK1/2 levels, and altered corticosteroid production. This finding suggests that ACTH regulates PHB1 in adrenocortical cells and plays a role in corticosteroid production, which was previously unknown.

Keywords: Adrenal Cortex, Adrenocorticotrophic Hormone, Cholesterol, Corticosterone, Mitochondria

1. Introduction

Corticosteroids are an important class of steroid hormones, which are synthesized and produced by the adrenal glands of vertebrates in response to steroidogenic stimuli[1]. There are two main classes of corticosteroids, glucocorticoids and mineralocorticoids. The importance of corticosteroids is evident from their wide-ranging essential functions in the body's physiology, including carbohydrate metabolism, stress response, regulation of salt balance, and immune functions[1,2]. In addition, adrenal glands produce adrenal androgens, such as Dehydroepiandrosterone (DHEA)[1,2]. The major regulator of glucocorticoids and adrenal androgen production is ACTH, produced by the pituitary gland and regulated by the hypothalamus and the central nervous system[3]. ACTH stimulation induces rapid synthesis and secretion of glucocorticoids and DHEA in adrenocortical cells. In addition, ACTH increases RNA and protein synthesis of steroidogenic enzymes and Steroidogenic Acute Regulatory protein (StAR)[4].

ACTH action on adrenocortical cells is mediated by its binding to cognate receptors (i.e., melanocortin-2 receptor), a member of the G-Protein Coupled Receptor (GPCR) family present on the plasma membrane. ACTH binding to its receptors leads to the activation of cAMP-

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PKA signaling pathways and several phosphoprotein kinases, phosphorylation, and acute regulation of StAR[4,5]. ACTH action increases free cholesterol availability for steroidogenesis by modulating proteins involved in intracellular cholesterol homeostasis, such as cholesterol uptake, cholesterol ester hydrolase, and cholesteryl ester synthase[4,5]. Subsequently, the StAR protein transports the free cholesterol from the cytoplasm through the Outer Mitochondrial Membrane (OMM); however, its further transport to the Inner Mitochondrial Membrane (IMM), where steroidogenesis begins, remains unclear.

Prohibitin-1 (PHB1) is a hallmark protein of the IMM, reportedly involved in various aspects of mitochondrial biology, including mitochondrial dynamics[6]. PHB1 and its homolog protein PHB2 form heterodimeric protein complexes in the IMM that are required for structural and functional integrity of the mitochondria[7]. Both PHBs belong to a group of proteins family, the PHB domain family, which are thought to function as lipid and protein scaffolds in the IMM that affect the lateral distribution of the membrane lipid and protein components[8,9].

We have been interested in understanding the roles and regulation of PHB1, including its cell type-specific functions, such as adipocytes and pancreatic beta cells[10,11]. Recently, using a transgenic mouse model of PHB1 (*Fabp4*-PHB1-Tg), we have shown that PHB1 plays a crucial role in lipid homeostasis, mediated through its mitochondrial functions[12]. Subsequent studies revealed that the male PHB1 transgenic mice exhibit significantly higher testosterone levels[13]. Further investigation showed that it was due to overexpression of PHB1 in Leydig cells, consistent with a report that *Fabp4* expressed in Leydig cells[14]. The role of PHB1 in Leydig cell steroidogenesis is further confirmed using PHB1-manipulated cells[13]. Collectively, this evidence suggested an essential role of PHB1 in cholesterol processing and testosterone production. However, the potential role of PHB1 in corticosteroidogenesis is not explored, or at least has not been reported despite its very high expression levels in the adrenal cortex[15]. As the basic framework of pituitary-derived tropic hormones (i.e., ACTH, FSH, and LH)-induced steroidogenesis in different steroidogenic tissues is very similar, including early cell signalling events leading to cholesterol transport to mitochondria and initiation of steroidogenesis, it is conceivable that PHB1 might play a role in ACTH-induced steroidogenesis in adrenocortical cells. Thus, we examined the effect of ACTH on PHB1 levels in model human HAC15 and mouse Y-1 adrenocortical cells[16-18]. In addition, we examined the effect of manipulating PHB1 levels in adrenocortical cells on ACTH-induced cellular responses. Here, we report that ACTH regulates PHB1 levels in adrenocortical cells, and PHB1 plays a role in glucocorticoid production.

2. Materials and Methods

2.1 Materials

Materials used in this study and their sources are provided in Table 1.

2.2 Methods

2.2.1 Cell Culture and Treatment of Adrenocortical Cells

The murine adrenocortical Y-1 cells were cultured in ATCC recommended F12-K media supplemented with FBS to a final concentration of 2.5%, horse serum to a final concentration of 15%, and 1% penicillin and streptomycin mix at 37° C in 5% CO_2 atmosphere. The human adrenocortical HAC15 cells were grown in ATCC recommended F12-Ham (DMEM/F12) culture media supplemented with 50 ml FBS and 5 mL ITS + Premix added in 500 mL media bottle and 1% penicillin and streptomycin mix at 37° C in 5% CO_2 atmosphere. Cells were plated overnight in 6-well plate at 0.3 x 10^6/well density. The growth media was replaced with serum-free media 6-8 hrs before the treatment. Cells were then treated with ACTH (15 nM) or dibutyryl cAMP (db-cAMP) (0.5 mM) for 2 hrs or various time points, as indicated/applicable. The dose and time of treatment were determined from previous studies[18-20].

2.2.2 Cell Transfection

The pCMV6-XL5 vector containing the human PHB1 clone was obtained from Origene Technologies[21,22]. Glycerol stocks of PHB1 shRNA and scrambled control shRNA (shControl) were purchased from Dharmacon Inc. The bacterial transformation, culture, and plasmid preparation were performed as described previously[13] or following the manufacturer's protocol. Cell transfection was performed using the X-tremeGENE HP DNA

Table 1. Reagents used in the experiments and their sources

Reagents	Sources (Catalogue #)
Y-1 cells	ATCC, Manassas, USA (CCL-79)
F-12K	Thermo Fischer Canada
Horse serum	Thermo Fischer Canada
Penicillin and streptomycin mix	Thermo Fischer Canada
Fetal bovine serum	Thermo Fischer Canada
HAC15 cells	ATCC, Manassas, USA (CRL-3301)
Insulin, human transferrin, and selenous acid (ITS) premix	Beckton Dickinson (354352)
DMEM/F12	Thermo Fischer Canada
ACTH	Sigma-Aldrich Canada
Db-cAMP	Sigma-Aldrich Canada
All antibodies	Cell Signaling Technology, Danvers, USA
pCMV6-XL5 PHB1	Origene Technology
shRNAs (shControl and shPHB1)	Dharmacon Incorporation.
X-tremeGENE HP DNA transfection reagent	Sigma-Aldrich Canada
Corticosterone DRG ELISA assay kit	Cedarlane Canada

ATCC – American type culture collection; ACTH – Adrenocorticotrophic hormone; Db-cAMP – Dibutyryl cyclic-adenosine mono phosphate; pCMV-XL5 PHB1 – Prohibitin-1 clone in human cytomegalovirus mammalian expression vector; shRNA – short hairpin ribonucleic acid (scrambled control and prohibitin-1); HAC15 – a clonal human adrenocortical cell line; Y-1 – a murine adrenocortical cell line; F-12K – Ham's *F-12K* (Kaighn's) Medium; DMEM - Dulbecco's Modified Eagle's Medium; ELISA - Enzyme-linked immunosorbent assay.

transfection reagent according to the manufacturer's protocol.

2.2.3 Western Immunoblotting

At each time point (following ACTH or db-cAMP treatment), cells were harvested separately and processed for the preparation of cell lysates, as described in our recent study[13]. Cells in control groups (without ACTH or db-cAMP treatment) were processed concurrently. Subsequently, each protein sample (20 µg each) was loaded for electrophoresis on 12% SDS-PAGE and electro-transferred to Polyvinylidene Difluoride (PVDF) membranes[13,16]. Then, the blots were blocked with 5% nonfat dry milk in TBS, 0.1% Tween-20 for 1 h, and incubated with respective primary antibodies overnight at 4^{0}C with gentle shaking. The following day, the membranes were washed in TBST for 3 x 10 min and incubated with horseradish peroxidase secondary antibody for 1 h at room temperature. Membranes were washed again in TBST (3 x 10 min), and immunoreactivity of protein bands was detected by chemiluminescence reagent[13,16]. The images of protein bands were captured with a ChemiDoc system (Bio-Rad Laboratories).

2.2.4 Transmission Electron Microscopy (TEM)

The TEM analysis of adrenocortical cells cultured under different experimental conditions was performed using a Philips CM10 at 80 kV at the Histomorphology & Ultrastructural Core Facility in the Department of Anatomy and Cell Sciences, University of Manitoba, as described previously[13,16]. Briefly, cells were harvested using 0.25% trypsin, followed by centrifugation at 1100 rpm for 5 min. The resulting cell pellets were fixed with 3% glutaraldehyde in 0.1 M Sorensen's buffer for 3 hours. Subsequently, the cells were resuspended in 5% sucrose in 0.1 M Sorensen's buffer and then embedded in EPON™ resin. Then, ultra-thin sections (100 nm) of

adrenocortical cells were stained with uranyl acetate and counter stained with lead citrate for TEM analysis[13,16].

2.2.5 Measurement of Corticosterone Levels

Corticosterone levels in Y-1 total cells were measured using a DRG ELISA kit (Catalogue # EIA-4164) with inter- and intra-assay coefficient variation (% CV) of 5.5–6.3 and 2.4–4.0, respectively. The assay was performed according to the manufacturer's instructions.

2.2.6 Quantifications and Statistical Analysis

Quantification of Lipid Droplets (LDs) and mitochondrial numbers in the transmission electron micrographs of adrenocortical cells, as well as protein band densities in immunoblots, were performed using ImageJ software (https://imagej.nih.gov/ij/)[16,23]. All statistical analyses were performed using GraphPad Prism 6.2 software (La Jolla, CA). The Student's *t*-test was performed for comparison between two experimental groups. Moreover, we performed an Analysis of Variance (ANOVA) with the Dunnett test to compare every mean to a control mean, whereas the Tukey test was performed for multiple comparisons[24]. A *p*-value of <0.05 was considered statistically significant in all analyses. Data are presented as means ± the Standard Error of the Mean (SEM). All experiments were repeated at least 3 times.

3. Results

3.1 ACTH Regulates PHB1 Levels in Adrenocortical Cells

Recently, we have reported that PHB1 is an LH-regulated protein in Leydig cells (steroidogenic cells in the testis) and plays a role in testosterone production[13]. This finding prompted us to investigate if ACTH regulates PHB1 protein levels in adrenocortical cells, since the basic framework of steroidogenesis is similar in steroidogenic cells in the testis and the adrenal glands[16,17]. The stimulation of human HAC15 and mouse Y-1 adrenocortical cells with ACTH increased PHB1 protein levels in a time-dependent manner, especially an acute upregulation was observed in both cell lines at early time points (e.g. 1 h) (Figure 1 A, C). To further confirm the acute regulation of PHB1 protein in adrenocortical cells, we repeated the experiment with dibutyryl cyclic-AMP (db-cAMP), a cell permeable analogue of cAMP, which mimics ACTH action in adrenocortical cells[13,16,25]. As expected, a similar and more sustained increase in PHB1 levels was observed in response to db-cAMP in both adrenocortical cell lines (Figure 1 B, D). Our finding of an acute rise in PHB1 levels earlypoint following ACTH or db-cAMP stimulation compelled us to examine whether PHB1 is regulated at the translational level in adrenocortical cells. For this, we stimulated HAC15 and Y-1 cells with db-cAMP in the presence of Cycloheximide (CHX), a protein synthesis inhibitor[13]. An inhibition of the acute increase in PHB1 protein level (in response to db-cAMP stimulation) was observed in both cell types (Figure 1 E, F), suggesting that PHB1 is regulated at the translational level in adrenocortical cells. This data suggests that ACTH regulates PHB1 protein levels in adrenocortical c ells.

3.2 Altering PHB1 Levels in Adrenocortical Cells Affects Steroidogenic Features

PHB1 plays a role in mitochondrial biology and lipid homeostasis[6-9,26-29], which appears to be interrelated. For example, we have shown that PHB1 overexpression in adipocytes increases mitochondrial biogenesis and adipose tissue mass[12], whereas PHB1 knockdown in adipocytes was found to decrease adipose tissue mass[30]. Moreover, we have found that manipulation of the PHB1 level or its phosphorylation-dependent function in Leydig cells affects cholesterol homeostasis and mitochondrial features[13]. Therefore, we examined the effect of PHB1 overexpression and knockdown on the ultrastructure (e.g., lipid droplets and mitochondrial features) of adrenocortical cells using Transmission Electron Microscopy (TEM) after confirming intended changes in PHB1 levels by immunoblotting (Figure 2). An increase in mitochondrial ($p < 0.05$) and lysosome numbers ($p < 0.01$) were observed in adrenocortical cells overexpressing PHB1 compared with vector transfected control cells (Figure 3). In contrast, shRNA-mediated PHB1 knockdown increased lipid droplet number and size ($p < 0.01$), as well decreased mitochondrial number ($p < 0.05$) and caused structural abnormality (e.g., poor mitochondrial cristae organization) (Figure 4). However, no difference was found in lysosome number and size (not shown). In addition, a sign of autophagy/lipophagy (e.g., autophagosome/lipophagosome) were observed in

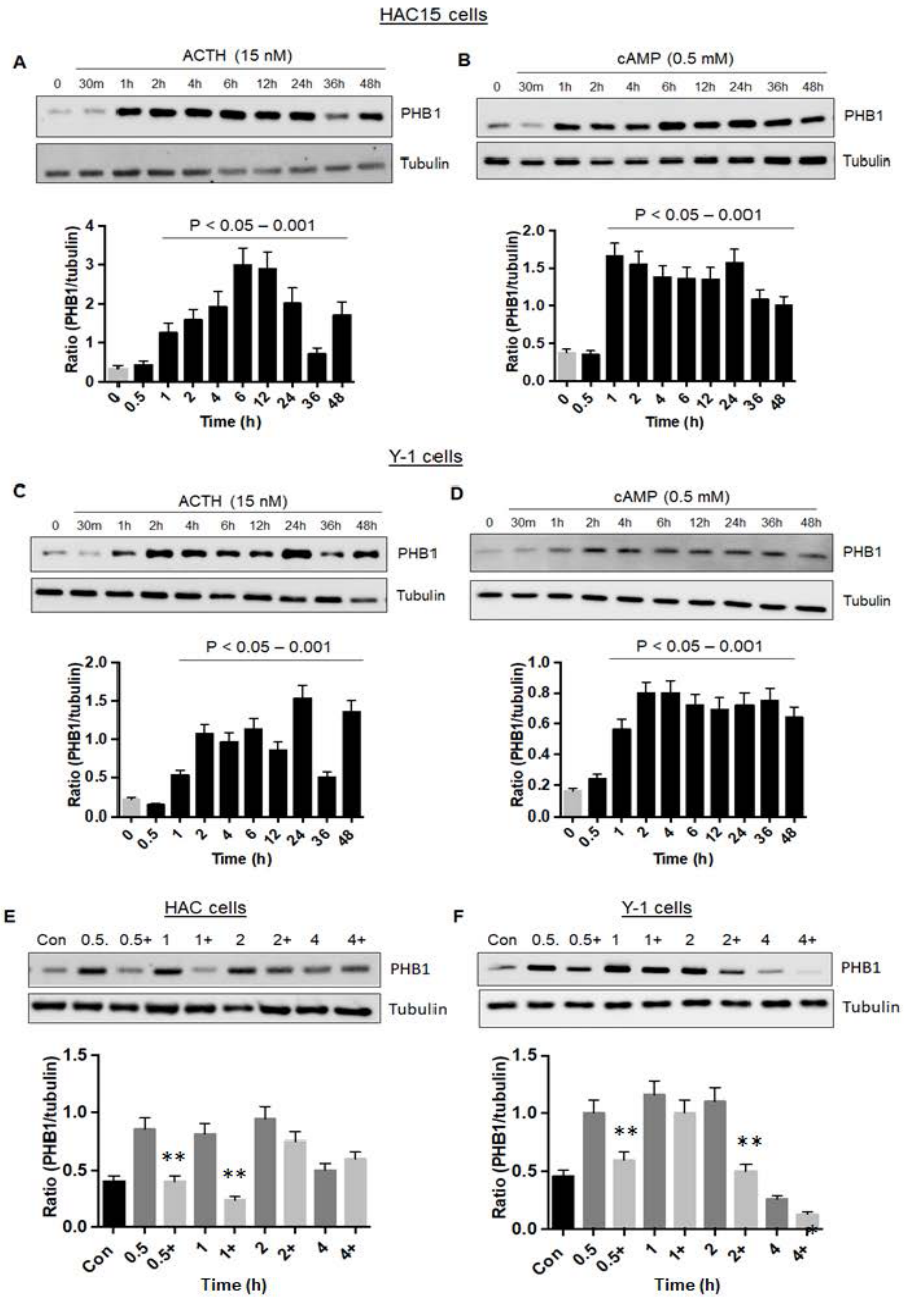

Figure 1. PHB1 is an ACTH-regulated protein in adrenocortical cells. (A-D) Representative immunoblots showing time-dependent effect of ACTH (A and C) or db-cAMP (B and D) on PHB1 expression levels in HAC15 and Y-1 adrenocortical cells. Tubulin blot is shown as a loading control. Quantification of band intensities are shown with histograms (lower panel). Data are presented as mean ± SEM (n=3). (E and F) Representative immunoblots showing the effect of cycloheximide (CHX) treatment on ACTH-induced changes in PHB1 levels in HAC15 and Y-1 adrenocortical cells. *p <0.05, **p < 0.01 between ACTH and ACTH + CHX.

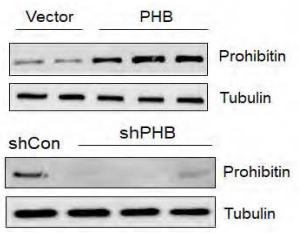

Figure 2. Representative immunoblots showing validation of PHB1 overexpression and knockdown in Y-1 adrenocortical cells. Tubulin blots are shown as loading control (n = 3).

adrenocortical cells after PHB1 knockdown (Figure 3). Taken together, this data indicates that PHB1 plays a role in lipid/cholesterol homeostasis and processing, and in the regulation of mitochondrial features in adrenocortical cells.

3.3 Manipulation of PHB1 Levels in Adrenocortical Cells Affects ACTH-Induced p-PKA and p-ERK1/2 Levels

Many studies have shown that PHB1, in addition to role in mitochondrial biology and lipid homeostasis, plays a role in the modulation of membrane signaling. For example, we have shown that the phosphorylation of PHB1 plays a role in PI3K-Akt and MAPK-ERK1/2 signaling[10,21,22]. Moreover, PHB1 has been reported to

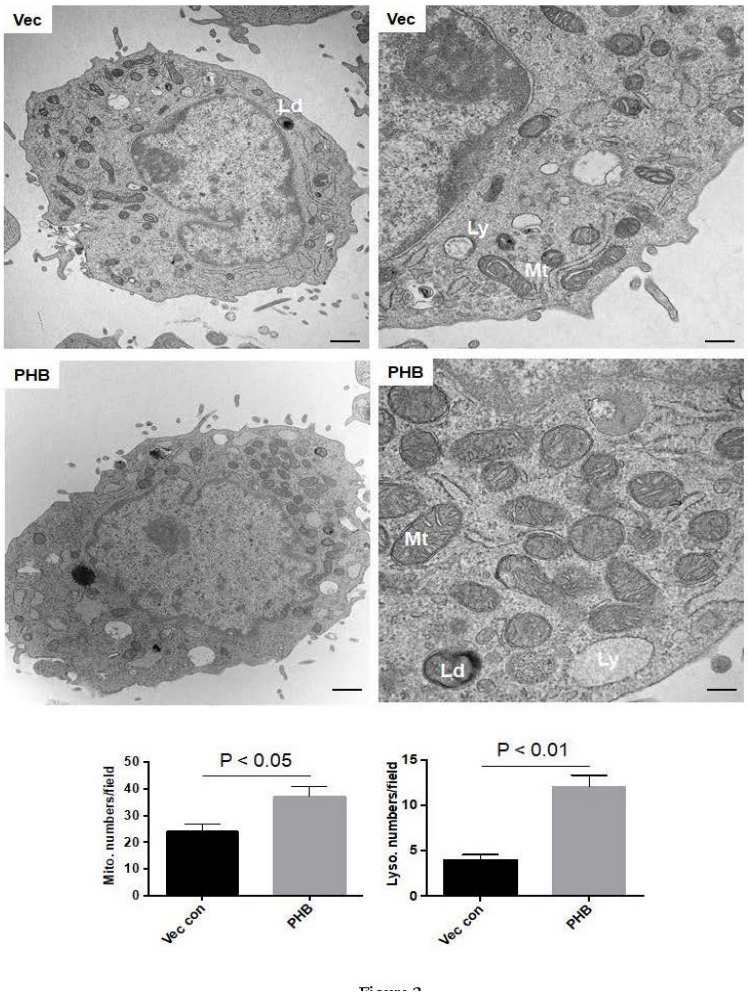

Figure 3. Transmission electron micrographs showing the effect of PHB1 overexpression on mitochondrial and lysosomal characteristics in Y-1 adrenocortical cells (upper panel and middle panel). Histograms showing quantification of mitochondrial numbers and lysosome numbers (lower panel). Data are presented as mean ± SEM (7-10 cells were counted in each group). Mt – mitochondria, Ld – lipid droplets and Ly – lysosomes.

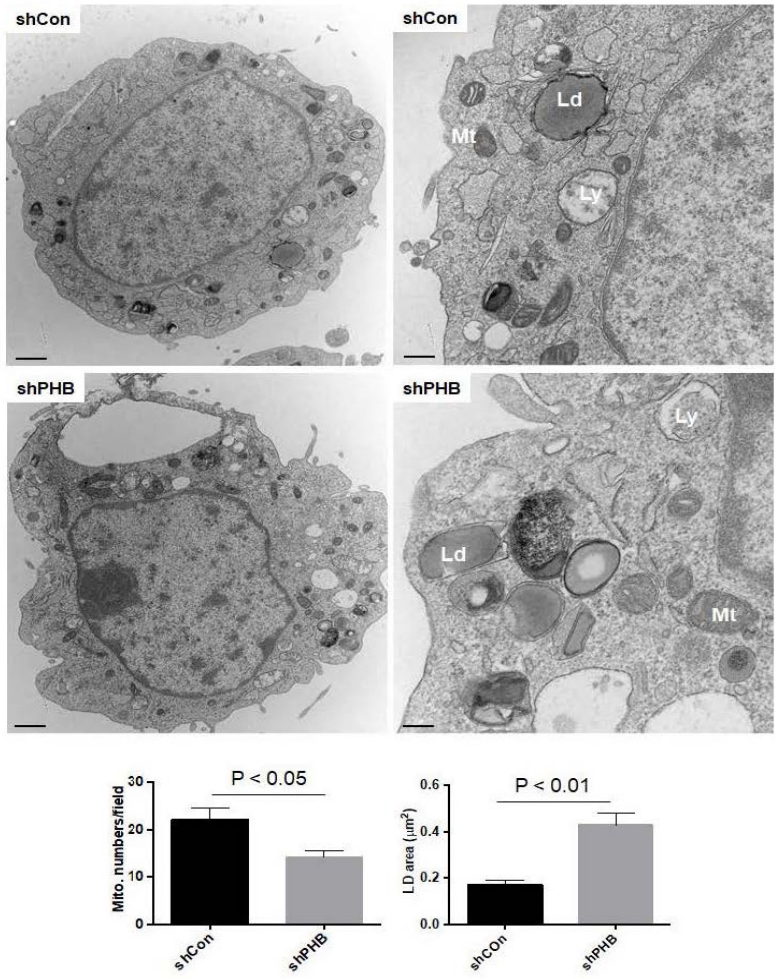

Figure 4. Transmission electron micrographs showing the effect of shRNA-mediated PHB1 knockdown on mitochondrial and lipid droplet characteristics in Y-1 adrenocortical cells (upper panel and middle panel). Histograms showing quantification of mitochondrial numbers and lipid droplet area (lower panel). Data are presented as mean ± SEM (n =3). Mt – mitochondria; Ld – lipid droplets; Ly – lysosomes.

undergo phosphorylation in many cell types in response to growth factors and hormonal stimulation[21,22,31-34] and antigen-stimulated immune signaling pathways in different immune cell types[35,36]. Thus, we examined the phosphorylation levels of PKA and ERK1/2 in PHB1 manipulated adrenocortical cells because of their established roles in mediating the effects of trophic hormones in steroidogenic cells[13,17]. First, we examined the cAMP-PKA signaling pathway because of its central role in mediating the ACTH response in adrenocortical cells[37]. For this, we determined the phospho-PKA (p-PKA) levels in adrenocortical cell lysates by immunoblotting using a phospho-specific antibody. An increase in p-PKA level was found in cells overexpressing PHB1 compared to control experimental group (Figure 5), which was abrogated after PHB1 knockdown (Figure 5). We also examined the phospho-ERK1/2 (p-ERK1/2) levels in PHB1-manipulated adrenocortical cells because of their role in steroidogenesis[13,17], and several studies have reported that PHB1 modulates MAPK-ERK1/2 signaling in different cell/tissue types[10,32,33]. The p-ERK1/2 level was also increased in PHB1 overexpressing cells, which

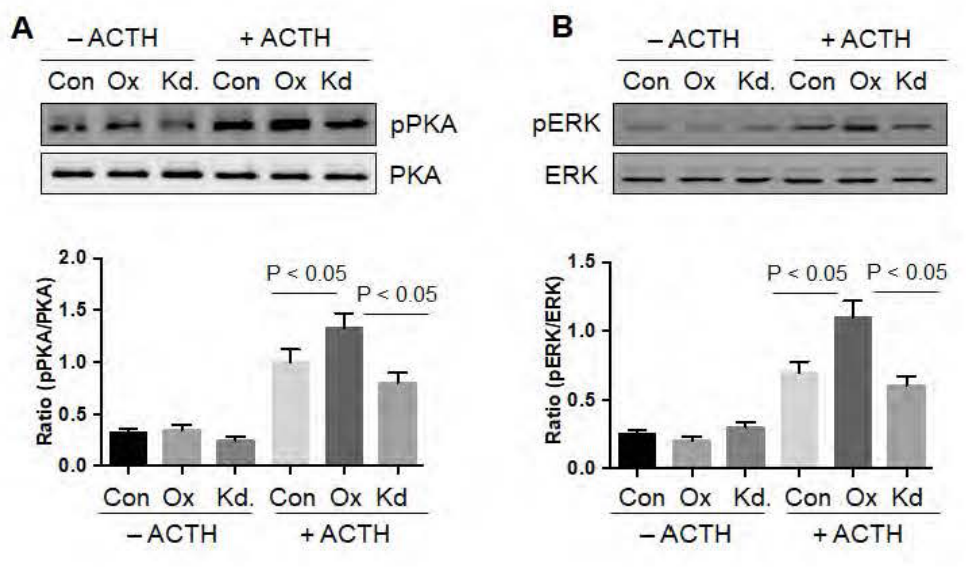

Figure 5. Immunoblots showing the effect of PHB1 overexpression on the phosphorylation of PKA (A) and ERK (B) with/without steroidogenic stimulation (upper panel) and their reversal by PHB knockdown. Quantification of band intensities are shown with histograms. Data are presented as mean ± SEM (n =3). Con – control; Ox – PHB1 overexpression; Kd – PHB1 knockdown.

was reversed by PHB1 knockdown (Figure 5). Together, this data suggests a potential involvement of p-PKA and p-ERK1/2 in mediating PHB1's role in adrenocortical cells.

3.4 PHB1 Plays a Role in Glucocorticoid Production by Adrenocortical Cells

Finally, to determine the functional relevance of PHB1 in adrenocortical cells, we examined the effect of

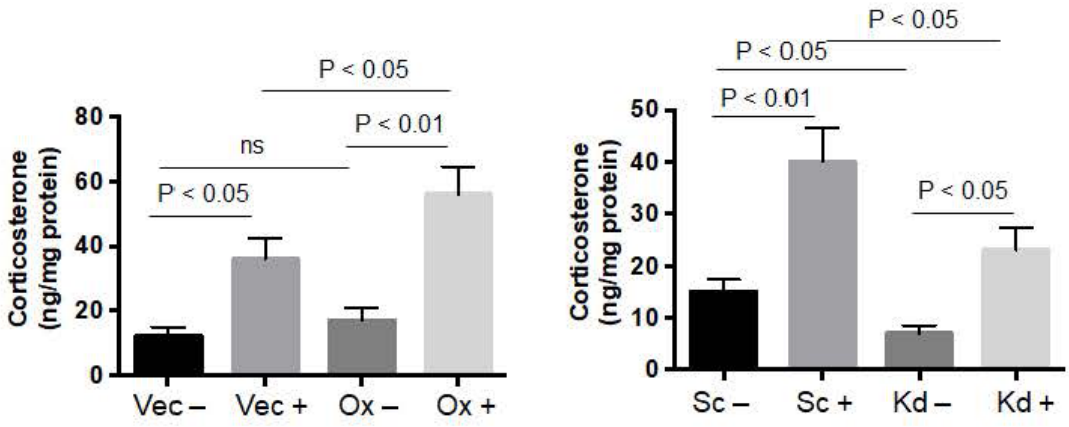

Figure 6. Histograms depict the effect of PHB1 overexpression and knockdown on corticoid production from adrenocortical cells in response to db-cAMP. Data are presented as mean ± SEM (n =3). – and + indicate without or with db-cAMP stimulation, respectively. Vec – vector control; Ox – PHB1 overexpression; Kd – shRNA-mediated PHB1 knockdown; Sc – scrambled control shRNA.

PHB1 overexpression and knockdown on db-cAMP-induced glucocorticoid production by adrenocortical cells. PHB1 overexpression increased db-cAMP-induced corticosterone production ($p < 0.05$; Figure 6), whereas its knockdown decreased db-cAMP-induced corticosterone production ($p < 0.05$; Figure 6) respective to their control groups. In addition, a significant decrease in corticosterone production was observed after PHB1 knockdown under basal condition (i.e., independent of db-cAMP stimulation) when compared with its control group ($p < 0.05$) (Figure 6). Together, this data suggests that PHB1 plays a role in glucocorticoid production by adrenocortical cells.

4. Discussion

Here, we report that PHB1 is an ACTH-regulated protein found in adrenocortical cells and plays a role in glucocorticoid production. This protein seems to play a role in cholesterol homeostasis, mitochondrial biology, and modulating steroidogenic cell signaling pathways. Thus, we report a previously unknown function of PHB1 in mediating ACTH-induced steroidogenesis in adrenocortical cells. Our findings in adrenocortical cells from two different species (i.e., human and mouse), along with our recent discovery of PHB1 role in testosterone production in Leydig cells[13], would suggest that its role in steroidogenesis is likely conserved in different steroidogenic cell types.

Abundant mitochondria and cholesterol containing Lipid Droplets (LDs), the two characteristic features of steroidogenic cells, are relatively more profound in glucocorticoid producing cells of the adrenal cortex amongst all steroidogenic cell types[1,2]. Our findings of substantial changes in LD areas and mitochondrial numbers in PHB1-manipulated adrenocortical cells imply that PHB1 plays a role in intracellular cholesterol homeostasis and mitochondrial dynamics. We have recently reported that PHB1 and its homolog protein PHB2 contains Cholesterol-Recognition Amino Acid Consensus (CRAC) motifs and binds cholesterol[13]. Moreover, we have previously shown that PHB1 contains lipid-binding motifs[22], and many PHB family members have been reported to bind cholesterol and be involved in lipid homeostasis at the organismic level[38-40]. In addition, PHB1 interacts with CD36 (a SR-B1-related protein) and plays a role in adipocyte lipid transport[30]. Moreover, PHB1 has been reported to be present in LD membrane[41]. Thus, the role of PHB1 in cholesterol homeostasis may include cholesterol uptake by steroidogenic cells, its storage in LDs, and transport to the mitochondria for subsequent utilization in steroidogenesis. Notably, the mechanism by which cholesterol is transported from the OMM to the IMM is unclear. Our findings of an IMM protein PHB1 (which forms a large heterodimeric protein complex with PHB2 spanning IMM) in two different steroidogenic cell types are a step forward. Importantly, an alteration in LD and mitochondrial features in PHB1 manipulated adrenocortical cells under basal conditions (i.e., independent of ACTH stimulation) signify that these changes are due to adrenocortical cell-specific alterations of PHB1. The manipulation of PHB1 levels in adrenocortical cells may change adrenocortical cell-specific attributes pertaining to steroidogenesis and ACTH-induced cell signaling events.

The findings, along with current knowledge in literature[11,12,30], indicate that the mitochondrial attributes of PHB1 contribute to the cell type-specific functions of PHB1 in lipid homeostasis in adipocytes[12,30] and steroidogenic cells[13], which further supports our conclusion that PHB1 plays a multifaceted role in adrenocortical cells. Altered cholesterol homeostasis and reduced glucocorticoid production from adrenocortical cells in response to PHB1 knockdown strengthens the role of PHB1 in corticosteroidogenesis. A better understanding of the relationship between cholesterol and PHB1 in steroidogenesis is anticipated to shed light on the relationship between cell-extrinsic and -intrinsic factors and events involved in steroidogenesis.

In this study, we have used only *in vitro* models. Further investigations using *in vivo* experimental approaches, such as adrenocortical cell-specific transgenic mouse models of PHB1, are necessary to confirm the physiological and pathological significance of our findings. However, the findings of the present study are consistent with our recent discovery in transgenic mouse models of PHB1 in testosterone production *in vivo*[13]. The molecular mechanisms involved in the interplay between PHB1 and cholesterol in steroidogenesis, including cell compartment specific functions are yet to be determined. Future studies focusing on these issues will enhance our understanding of PHB1's role in the interplay between

mitochondria and cholesterol in the biosynthesis and production of steroid hormones, a fundamental aspect of mammalian physiology.

5. Acknowledgment

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6. References

1. Carroll TB, Aron DC, Findling JW, Tyrrell JB. Glucorticoids and adrenal androgens. In: Gardner D and Shoback D (eds), Greenspan's Basic and Clinical Endocrinology (9th). McGraw-Hill Companies; 2011. p. 285-327.
2. Stewart PM, Krone N. The adrenal cortex. In: Melmed S, Polonsky KS, P. Reed Larsen PR, Kronenberg HM (eds), Williams Textbook of Endocrinology (12th ed). Saunders Elsevier Canada; 2011. p. 479-544. https://doi.org/10.1016/B978-1-4377-0324-5.00015-8
3. Spencer RL, Deak T. A user's guide to HPA axis research. Physiol Behav. 2017; 178:43-65. https://doi.org/10.1016/j.physbeh.2016.11.014
4. Lightman SL, Birnie MT, Conway-Campbell BL. Dynamics of ACTH and cortisol secretion and implications for disease. Endocr Rev. 2020; 41. https://doi.org/10.1210/endrev/bnaa002
5. Li LA, Xia D, Wei S, J. Hartung, Ru-Qian Zhao. Characterization of adrenal ACTH signaling pathway and steroidogenic enzymes in Erhualian and Pietrain pigs with different plasma cortisol levels. Steroids. 2008; 73:806-14. https://doi.org/10.1016/j.steroids.2008.03.002
6. Richter-Dennerlein R, Korwitz A, Haag M, Tatsuta T, Dargazanli S, Baker M, *et al*. DNAJC19, a mitochondrial cochaperone associated with cardiomyopathy, forms a complex with prohibitins to regulate cardiolipin remodeling. Cell Metab. 2014; 20:158-71. https://doi.org/10.1016/j.cmet.2014.04.016
7. Osman C, Merkwirth C, Langer T. Prohibitins and the functional compartmentalization of mitochondrial membranes. J Cell Sci. 2009; 122:3823-30. https://doi.org/10.1242/jcs.037655
8. Christie DA, Lemke CD, Elias IM, Chau LA, Kirchhof MG, Li B, *et al*. Stomatin-like protein 2 binds cardiolipin and regulates mitochondrial biogenesis and function. Mol Cell Biol. 2011; 31:3845-56. https://doi.org/10.1128/MCB.05393-11
9. Osman C, Haag M, Potting C, Rodenfels J, Dip PV, Wieland FT, *et al*. The genetic interactome of prohibitins: coordinated control of cardiolipin and phosphatidylethanolamine by conserved regulators in mitochondria. J Cell Biol. 2009; 184:583-96. https://doi.org/10.1083/jcb.200810189
10. Ande SR, Xu Z, Gu Y, Mishra S. Prohibitin has an important role in adipocyte differentiation. Int J Obes (Lond). 2012; 36:1236-44. https://doi.org/10.1038/ijo.2011.227
11. Lee JH, Nguyen KH, Mishra S, Nyomba BL. Prohibitin is expressed in pancreatic beta-cells and protects against oxidative and proapoptotic effects of ethanol. FEBS J. 2010; 277(2):488-500. https://doi.org/10.1111/j.1742-4658.2009.07505.x
12. Ande SR, Nguyen KH, Padilla-Meier GP, Wahida W, Nyomba BGL, Mishra S. Prohibitin overexpression in adipocytes induces mitochondrial biogenesis, leads to obesity development, and affects glucose homeostasis in a sex-specific manner. Diabetes. 2014; 63:3734-41. https://doi.org/10.2337/db13-1807
13. Bassi G, Mishra S. Prohibitin-1 plays a regulatory role in Leydig cell steroidogenesis. iScience. 2022; 25. https://doi.org/10.1016/j.isci.2022.104165
14. O'Hara L, McInnes K, Simitsidellis I, Morgan S, Atanassova N, Slowikowska-Hilczer J, *et al*. Autocrine androgen action is essential for Leydig cell maturation and function, and protects against late-onset Leydig cell apoptosis in both mice and men. FASEB J. 2015; 29(3):894-910. https://doi.org/10.1096/fj.14-255729
15. Uhlén M, Fagerberg L, Hallström BM, et al. Tissue-based map of the human proteome. Science. 2015; 23;347(6220):1260419.
16. Bassi G, Sidhu SK, Mishra S. The intracellular cholesterol pool in steroidogenic cells plays a role in basal steroidogenesis. J Steroid Biochem Mol Biol. 2022; 220. https://doi.org/10.1016/j.jsbmb.2022.106099
17. Bassi G, Sidhu SK, Mishra S. The expanding role of mitochondria, autophagy and lipophagy in steroidogenesis. Cells. 2021; 10. https://doi.org/10.3390/cells10081851
18. Schimmer BP, Cordova M. Corticotropin (ACTH) regulates alternative RNA splicing in Y1 mouse adrenocortical tumor cells. Mol Cell Endocrinol. 2015; 408:5-11. https://doi.org/10.1016/j.mce.2014.09.026

19. Ma R, Zhai L, Xu X, Lou T, *et al*. Ginsenoside Rd attenuates ACTH-induced corticosterone secretion by blocking the MC2R-cAMP/PKA/CREB pathway in Y1 mouse adrenocortical cells. Life Sci. 2020; 245. https://doi.org/10.1016/j.lfs.2020.117337
20. Parmar J, Key RE, Rainey WE. Development of an adrenocorticotropin-responsive human adrenocortical carcinoma cell line. J Clin Endocrinol Metab. 2008; 93:4542-6. https://doi.org/10.1210/jc.2008-0903
21. Ande SR, Gu Y, Nyomba BL. Insulin induced phosphorylation of prohibitin at tyrosine 114 recruits Shp1. Biochim Biophys Acta. 2009; 1793:1372-8. https://doi.org/10.1016/j.bbamcr.2009.05.008
22. Ande SR, Mishra S. Prohibitin interacts with phosphatidylinositol 3,4,5-triphosphate (PIP3) and modulates insulin signaling. Biochem Biophys Res Commun. 2009; 390:1023-8. https://doi.org/10.1016/j.bbrc.2009.10.101
23. Xu YXZ, Ande SR, Mishra S. Gonadectomy in Mito-Ob mice revealed a sex-dimorphic relationship between prohibitin and sex steroids in adipose tissue biology and glucose homeostasis. Biol Sex Differ. 2018; 9. https://doi.org/10.1186/s13293-018-0196-4
24. Ande SR, Nguyen KH, Grégoire Nyomba BL, Mishra S. Prohibitin-induced, obesity-associated insulin resistance and accompanying low-grade inflammation causes NASH and HCC. Sci Rep. 2016; 6. https://doi.org/10.1038/srep23608
25. Kimura E, Armelin HA. Phorbol ester mimics ACTH action in corticoadrenal cells stimulating steroidogenesis, blocking cell cycle, changing cell shape, and inducing c-fos proto-oncogene expression. J Biol Chem. 1990; 265:3518-21. https://doi.org/10.1016/S0021-9258(19)39799-6
26. Tatsuta T, Model K, Langer T. Formation of membrane-bound ring complexes by prohibitins in mitochondria. Mol Biol Cell. 2005; 16:248-59. https://doi.org/10.1091/mbc.e04-09-0807
27. Steglich G, Neupert W, Langer T. Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria. Mol Cell Biol. 1999; 19:3435-42. https://doi.org/10.1128/MCB.19.5.3435
28. Anand R, Wai T, Baker MJ, Kladt N, Schauss AC, Rugarli E, *et al*. The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. J Cell Biol. 2014; 204:919-29. https://doi.org/10.1083/jcb.201308006
29. Wai T, Saita S, Nolte H, Müller S, König T, Richter-Dennerlein R, *et al*. The membrane scaffold SLP2 anchors a proteolytic hub in mitochondria containing PARL and the i-AAA protease YME1L. EMBO Rep. 2016; 17:1844-56. https://doi.org/10.15252/embr.201642698
30. Gao Z, Daquinag AC, Fussell C, Djehal A, Désaubry L, Kolonin MG. Prohibitin inactivation in adipocytes results in reduced lipid metabolism and adaptive thermogenesis impairment. Diabetes. 2021; 70:2204-12. https://doi.org/10.2337/db21-0094
31. Rajalingam K, Wunder C, Brinkmann V, Churin Y, Hekman M, Sievers C. Prohibitin is required for Ras-induced Raf-MEK-ERK activation and epithelial cell migration. Nat Cell Biol. 2005; 7:837-43. https://doi.org/10.1038/ncb1283
32. Chowdhury I, Thomas K, Zeleznik A, Thompson WE. Prohibitin regulates the FSH signaling pathway in rat granulosa cell differentiation. J Mol Endocrinol. 2016; 56:325-36. https://doi.org/10.1530/JME-15-0278
33. Chowdhury I, Thompson WE, Welch C. Thomas K, Matthews R. Prohibitin (PHB) inhibits apoptosis in rat Granulosa Cells (GCs) through the extracellular signal-regulated kinase 1/2 (ERK1/2) and the Bcl family of proteins. Apoptosis. 2013; 18:1513-25. https://doi.org/10.1007/s10495-013-0901-z
34. Wang Q, Leader A, Tsang BK. Inhibitory roles of prohibitin and chemerin in FSH-induced rat granulosa cell steroidogenesis. Endocrinology. 2013; 154:956-67. https://doi.org/10.1210/en.2012-1836
35. Zhang J, Sun Z, Wu Q, Shen J. Prohibitin 1 interacts with signal transducer and activator of transcription 3 in T-helper 17 cells. Immunol Lett. 2010; 219:8-14. https://doi.org/10.1016/j.imlet.2019.12.008
36. Yurugi H, Tanida S, Akita K, Ishida A, Toda M, Nakada H. Prohibitins function as endogenous ligands for Siglec-9 and negatively regulate TCR signaling upon ligation. Biochem Biophys Res Commun. 2013; 434:376-81. https://doi.org/10.1016/j.bbrc.2013.03.085
37. Forti FL, Schwindt TT, Moraes MS, Eichler CB, Armelin HA. ACTH promotion of p27(Kip1) induction in mouse Y1 adrenocortical tumor cells is dependent on both PKA activation and Akt/PKB inactivation. Biochemistry. 2002; 41:10133-40. https://doi.org/10.1021/bi0258086
38. Ande SR, Mishra S. Palmitoylation of prohibitin at cysteine 69 facilitates its membrane translocation and interaction with Eps 1065 15 homology domain protein 2 (EHD2). Biochem Cell Biol. 2010; 88:553-8. https://doi.org/10.1139/O09-177

39. Huber TB, Schermer B, Müller RU, Höhne M, Bartram M, Calixto A, *et al*. Podocin and MEC-2 bind cholesterol to regulate the activity of associated ion channels. Proc Natl Acad Sci USA. 2006; 103:17079-86. https://doi.org/10.1073/pnas.0607465103
40. Browman DT, Resek ME, Zajchowski LD, Robbins SM. Erlin-1 and erlin-2 are novel members of the prohibitin family of proteins that define lipid-raft-like domains of the ER. J Cell Sci. 2006; 119:3149-60. https://doi.org/10.1242/jcs.03060
41. Brasaemle DL, Dolios G, Shapiro L, Wang R. Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3-L1 adipocytes. J Biol Chem. 2004; 279: 46835-42. https://doi.org/10.1074/jbc.M409340200