Journal of Biomedical and Translational Research
pISSN 2508-1357 eISSN 2508-139X

Original Article
J Biomed Transl Res 2023;24(4):117-126
https://doi.org/10.12729/jbtr.2023.24.4.117

Loss of progesterone receptor membrane component 1 decreases hepatic hepcidin levels in animal model

Moeka Mukae, Kang Ju Jeong, Eui-Ju Hong*

College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Korea

Received: Nov 17, 2023
Revised: Dec 11, 2023
Accepted: Dec 12, 2023

***Corresponding author**
Eui-Ju Hong
College of Veterinary Medicine,
Chungnam National University,
Daejeon 34134, Korea
Tel: +82-42-821-6781
E-mail: ejhong@cnu.ac.kr

ORCID
Moeka Mukae
https://orcid.org/0009-0007-6541-9936
Kang Ju Jeong
https://orcid.org/0009-0006-0826-4571
Eui-Ju Hong
https://orcid.org/0000-0001-9640-8841

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Acknowledgements
This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R1I1A2042991).

Ethics Approval
All mouse experiments were approved and performed in accordance with the Chungnam Facility Animal Care Committee (202209A-CNU-192).

Abstract

Iron is an essential nutrient for mammalian cells. Most iron absorption occurs in the duodenal epithelial cells and is regulated by hepcidin, which is produced and secreted in the liver. High hepcidin levels can cause iron deficiency anemia due to iron absorption failure. Inside the cell, iron conjugates with a porphyrin ring and is placed with an iron coordinated to heme. One of the heme-binding proteins, known as progesterone receptor membrane component 1 (Pgrmc1), is a non-canonical progesterone receptor associated with diverse molecular gene regulation. Previous studies showed that Pgrmc1 is related to iron homeostasis via hepcidin; however, these mechanisms remain to be elucidated. In the present study, to investigate the role of Pgrmc1 in mammalian iron metabolism, we introduced *Pgrmc1* knockout (KO) mice and performed molecular biological analyses using qPCR and western blotting. Pgrmc1 deficiency decreased *Hamp* mRNA expression and hepcidin protein levels. However, Pgrmc1 deficiency failed to decrease *Hamp* transcript expression and hepcidin protein levels in *siPGRMC1*-transfected HepG2 cells and primary *Pgrmc1* KO hepatocytes, respectively. PGRMC1 knockdown cells revealed low *HAMP* mRNA levels upon cyclic AMP (cAMP) activation, suggesting that PGRMC1 promotes *HAMP* mRNA transcription via cAMP activation. It has been implicated that hepatic Pgrmc1 cannot control hepcidin directly; however, the internal environment caused by the lack of Pgrmc1 may potentially cause low hepcidin levels.

Keywords: Pgrmc1; hepcidins; iron; cyclic AMP; HAMP

INTRODUCTION

Anemia is caused by abnormal hematopoietic cells, lack of vitamins and minerals in red blood cells, high vitamin demand due to pregnancy and lactation, and chronic bleeding due to digestive disease [1]. Clinically, anemia can cause general fatigue, hair loss, and mental illness. Moreover, the burden on the heart due to the lack of hemoglobin is related to cardiomyopathy [2]. While the prevalence of anemia is increasing annually in developed countries, the increase in anemia-related deaths is a problem that cannot been neglected in recent years [3]. Chronic iron deficiency can lead to anemia as iron is a cofactor for physiological proteins and is indispensable for life cycle activities, such as carrying oxygen to the mitochondria [4]. Some iron-regulating factors in the small intestinal epithelium, such as hepatocytes, macrophages, and the bone marrow, regulate iron

homeostasis [5]. Dietary ferric (Fe^{3+}) is reduced to ferrous (Fe^{2+}) by duodenal cytochrome B, located at the duodenal brush border membrane. Subsequently, Fe^{2+} passes through divalent metal transporter 1, which is expressed intracellularly at the surface cells [6, 7]. Alternatively, when there is a high hepcidin or low ferritin level in the epithelium, Fe^{2+} is oxidized again and stored in the epithelium as ferritin. Ferrous ions pass through ferroportin, which is the only channel to release iron into the blood flow. These ferrous ions get oxidized by hephaestin located at the basement membrane and then iron is transported to various organs [8].

Hepcidin, encoded by *Hamp*, is a peptide hormone produced and secreted by hepatocytes that influences duodenal epithelial cells and regulates dietary iron uptake. More specifically, hepcidin is a downregulatory factor that inhibits iron intake by binding with ferroportin and promoting the disassembly of ferroportin in the lysosome [9]. The function of hepcidin and the relationship between its controlling factors have been well assessed in previous studies [10]. One of the heme-binding proteins, known as progesterone receptor membrane component 1 (Pgrmc1), is a non-canonical progesterone receptor associated with diverse molecular gene regulation. Pgrmc1 functions in a variety of cellular processes, including heme binding, drug metabolism, progesterone responsiveness, steroidogenesis, female fertility, lipid transport, insulin secretion, cancer, and anti-apoptosis [11–13]. When its expression is substantially higher in the liver, it might be contained that hepatic Pgrmc1 controls iron homeostasis. According to a previous study, progesterone increases *Hamp* mRNA expression by increasing Pgrmc1 in zebrafish and HepG2 cells [14]. While Pgrmc1 can regulate *Hamp* mRNA expression, the role of Pgrmc1 has not been studied in a mammalian model. Thus, to investigate whether Pgrmc1 regulates hepcidin in mice, we generated *Pgrmc1* knockout (KO) mice and conduct molecular biological analyses using livers.

MATERIALS AND METHODS

Animals

C57BL/6J wild-type (WT) and *Pgrmc1* KO mice (both 10-week-old; male) were housed in a temperature- and light-controlled facility at Chungnam National University and fed standard chow with water provided *ad libitum*. All mouse experiments were approved and performed in accordance with the guidelines of the Chungnam Facility Animal Care Committee (202209A-CNU-192). Before necropsy, WT or *Pgrmc1* KO mice were fasted for 18 hr and then fed for 5 hr under a general feeding regimen.

Chemicals

Insulin was purchased from WelGENE. Forskolin was purchased from Tokyo Chemical Industry.

RNA isolation, cDNA synthesis and q-PCR

RNA was isolated using the TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA), chloroform (Sigma-Aldrich, St. Louis, MO, USA), and isopropanol (Kanto Chemical,

https://doi.org/10.12729/jbtr.2023.24.4.117

Tokyo, Japan) dissolved in water treated with diethyl pyrocarbonate (DEPC; Amresco, Solon, OH, USA). cDNA was acquired using a Reverse Transcriptase Kit (SmartGene, Lausanne, Switzerland). Specific primers, SYBR Green Q-PCR Master Mix with Low Rox (SmartGene), and Stratagene Mx3000P (Agilent Technologies, Santa Clara, CA, USA) were used to perform real-time PCR.

The human primers used were as follows: *PGRMC1* forward (AAA GGC CGC AAA TTC TAC GG), *PGRMC1* reverse (CCC AGT CAC TCA GAG TCT CCT), *Hamp* forward (TGA CCA GTG GCT CTG TTT TC), *Hamp* reverse (GAA AAT GCA GAT GGG GAA GT), *RPLP0* forward (TCG ACA ATG GCA GCA TCT AC), and *RPLP0* reverse (GCC TTG ACC TTT TCA GCA AG).

The mouse primers used were as follows: *Pgrmc1* forward (GGC AAG GTG TTC GAC GTG A), *Pgrmc1* reverse (GTC CAG GCA AAA TGT GGC AA), *Hamp* forward (CTG CCT GTC TCC TGC TTC TC), *Hamp* reverse (AGA TGC AGA TGG GGA AGT TG), *Fth1* forward (CGA GAT GAT GTG GCT CTG AA), *Fth1* reverse (GTG CAC ACT CCA TTG CAT TC), *Ftl1* forward (GGG CCT CCT ACA CCT ACC TC), *Ftl1* reverse (CTC CTG GGT TTT ACC CCA TT), *Bmp6* forward (TTC TTC AAG GTG AGC GAG GT), *Bmp6* reverse (TAG TTG GCA GCG TAG CCT TT), *IL-6* forward (CTG CAA GAG ACT TCC ATC CAG), *IL-6* reverse (AGT GGT ATA GAC AGG TCT GTT GG), *Rplp0* forward (GCA GCA GAT CCG CAT GTC GCT CCG), and *Rplp0* reverse (GAG CTG GCA CAG TGA CCT CAC ACG G).

Western blotting analysis

Liver, primary hepatocytes, and HepG2 cells were lysed in T-PER buffer (Thermo Fisher Scientific) and sonicated for protein extraction. Protein samples were quantified using the protein measurement solution (iNtRON, Seongnam, Korea) and ran in a 10%–12% SDS-PAGE after 5 min of boiling at 100°C. The gels were blotted at 300 mA for 80 min. PVDF membranes were blocked in 3% bovine serum albumin (BSA) for 15 min and incubated with primary antibodies diluted 1:2,500 in 3% BSA at 4°C for overnight. The following day, membranes were incubated with secondary antibodies diluted 1:2,500 in 5% skim milk at 4°C for overnight. The results were detected using ECL solution (Cyanagen, Bologna, Italy). The primary antibodies used were rabbit anti-Pgrmc1, rabbit anti-p38, rabbit anti-phospho-p38, rabbit anti-Akt, rabbit anti-phospho-Akt, rabbit anti-Hsp90 (Cell Signaling Technology, Danvers, MA, USA), rabbit anti-hepcidin, rabbit anti-Stat3, rabbit anti-phospho-Stat3, rabbit anti-Creb, and rabbit anti-phospho-Creb (ABclonal, Shanghai, China).

Cell culture

HepG2 cells obtained from the Korean Cell Line Bank. All the cell culture reagents were purchased from Welgene (Gyeongsan, Korea). HepG2 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 5% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/mol), streptomycin (100 μg/mL), ciprofloxacin (10 μg/mL), and gentamycin (50 μg/mL) at 37°C in 5% CO_2. HepG2 cells were washed with Dulbecco's Phosphate-Buffered Saline (DPBS) and incubated in a high-glucose medium (4,500 mg/dL, without FBS) for 3 hr.

Forskolin was added for 30 min or 1 hr after determining the treatment concentration.

For *PGRMC1* knockdown, HepG2 cells were transfected with negative control siRNA or *PGRMC1* siRNA and Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) in Opti-MEM (Thermo Fisher Scientific) medium for 72 hr. *PGRMC1* siRNA #1 (5'-CAGUACAGUCGC-UAGUCAA-3') and #2 (5'-CAGUUCACUUUCAAGUAUCA-U-3') were purchased from Bioneer (Daejeon, Korea).

Primary hepatocytes were maintained in a DMEM with 10% FBS, penicillin (100 U/mol) and streptomycin (100 μg/mL) at 37°C in 5% CO_2. Hepatocytes were washed with DPBS and incubated in high-glucose medium or high glucose and 10 nM insulin for 3 hr before harvest.

Primary hepatocyte isolation

Primary hepatocytes were isolated from WT and *Pgrmc1* KO mice. The animals were anesthetized with 2% isoflurane and 1% O_2, perfused with Ca^{2+} and Mg^{2+} free-Hanks Balanced Salt Solution (HBSS) containing 0.5 mM EDTA and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and then digested with HBSS containing Ca^{2+} and Liberase (Sigma-Aldrich). The livers were removed, rinsed with phosphate-buffered saline (PBS), and loosened in DMEM. After washing a few times, primary hepatocytes were seeded in cell culture plates and cultured in DMEM supplemented with 10% heat-inactivated FBS, penicillin (100 U/mol), and streptomycin (100 μg/mL) at 37°C in 5% CO_2. Hepatocytes were treated with 10 nM insulin for 3 hr after incubation in a high-glucose DMEM (containing 4,500 mg/dL glucose) without FBS for 3 hr.

RESULTS

Lack of Pgrmc1 decreased *Hamp* mRNA expression and hepcidin protein levels

To investigate the relationship between Pgrmc1 and hepcidin, male WT or *Pgrmc1* KO mice (n = 4) were observed. The mice were fasted for 18 hr and then fed a regular diet for 5 hr before necropsy. In terms of food intake status, *Pgrmc1* KO mice showed lower *Hamp* mRNA expression in the liver than WT mice. Ferritin heavy chain 1 (Fth1) and ferritin light chain 1 (Ftl1) are the major intracellular iron storage proteins. Their gene expression levels were lower than those in the WT mice (Fig. 1A). Moreover, hepcidin protein levels in *Pgrmc1* KO mice were lower than those in WT mice, suggesting that Pgrmc1 regulates hepcidin levels *in vivo* (Fig. 1B).

Pgrmc1 increases hepcidin *in vivo* independent of the BMP/SMAD pathway or inflammatory signaling

Hepcidin is regulated by the BMP-SMAD pathway or by an increase in inflammatory mediators, such as IL-6, JAK, and STAT3 signaling [15]. To determine how Pgrmc1 regulates hepcidin in the liver, we measured *Bmp6* and *IL-6* mRNA expression using qPCR. However, no significant differences were found between WT and *Pgrmc1* KO mice (Fig. 2A). Furthermore, the ratio of phospho-Stat3, an inflammatory marker expressed by IL-6 in hepcidin transcrip-

https://doi.org/10.12729/jbtr.2023.24.4.117

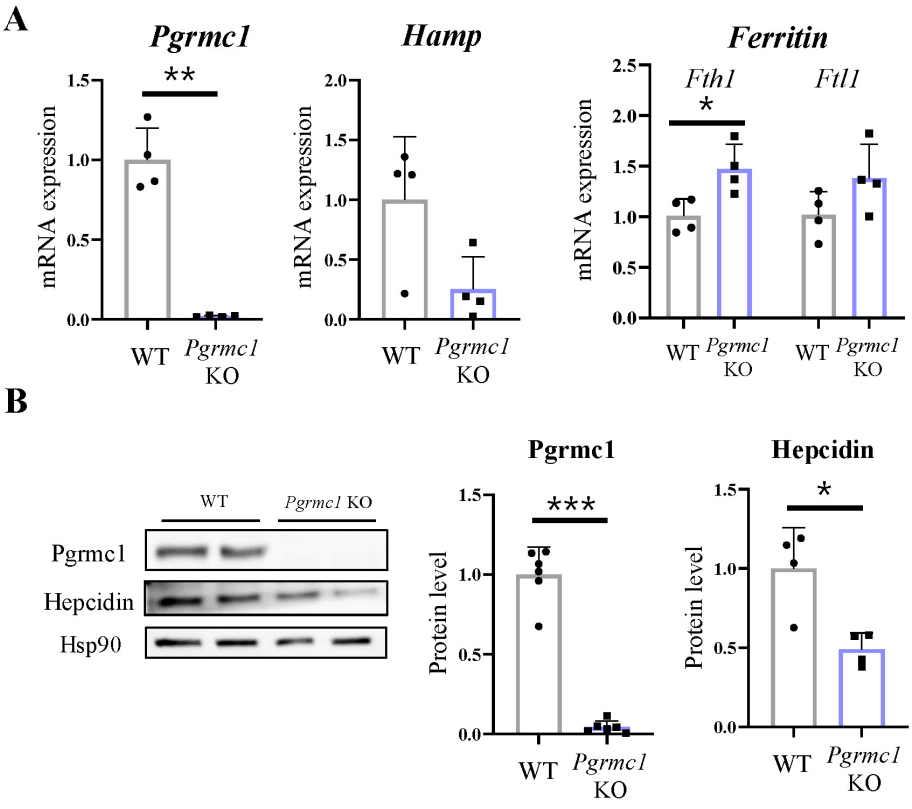

Fig. 1. Pgrmc1 and hepcidin expression in mice liver. (A) *Pgrmc1, Hamp* and *Ferritin* mRNA expression in the liver of WT or *Pgrmc1* KO mice in feeding status. *Rplp0* mRNA was used as an internal control. Values represent means ± S.D. * p<0.05. ** p<0.01. (B) Western blot analysis and quantification of Pgrmc1 and Hepcidin proteins in the liver of WT or *Pgrmc1* KO mice in feeding status. Hsp90 was used for an internal control. Values represent means ± S.D. * p<0.05. *** p<0.001. Total RNA was isolated from the liver of WT or *Pgrmc1* KO mice (n = 4). The protein analysis was repeated at least three times. WT, wild-type; KO, knockout.

tion signaling, was lower in WT mice than in *Pgrmc1* KO mice (Fig. 2B).

Hepatic Pgrmc1 fails to control hepcidin and *Hamp* mRNA expression in primary hepatocytes

To determine the factors that regulate hepcidin via Pgrmc1, we isolated primary hepatocytes from mice. The primary hepatocytes were incubated in high-glucose DMEM containing penicillin (100 U/mol), streptomycin (100 μg/mL), and 10% FBS for 24 hr. To assess the difference between primary hepatocytes of WT and *Pgrmc1* KO mice, we introduced primary hepatocytes into a high-glucose DMEM without FBS for 3 hr. However, no difference was found in hepcidin protein levels and *Hamp* mRNA expression between WT and *Pgrmc1* KO mice (Fig. 3A and B). Next, primary hepatocytes from WT and *Pgrmc1* KO mice were treated with 10 nM insulin for 3 hr after incubation in a high-glucose DMEM without FBS for 3 hr. While the ratio of phospho-Akt levels increased in the insulin-treated group, the hepcidin protein level failed to increase in the insulin-treated group. When *Hamp* mRNA expression was higher in the insulin-treated group, no difference was found between the WT and *Pgrmc1* KO mice (Fig. 3C). Additionally, *Pgrmc1* KO mice showed higher hepcidin protein levels than WT mice in

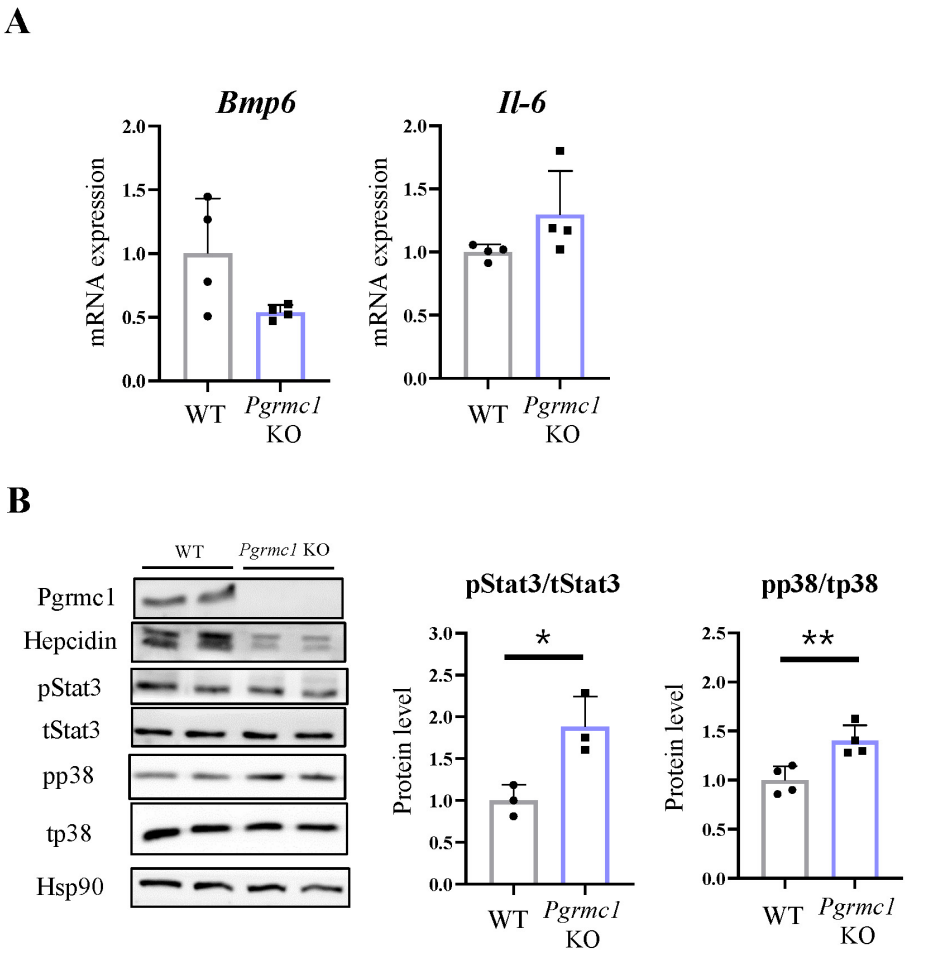

Fig. 2. Pgrmc1 did not involve BMP/SMAD pathway or inflammatory signaling in mice liver. (A) *Bmp6* and *IL-6* mRNA expression in the liver of WT or *Pgrmc1* KO mice in feeding status. *Rplp0* mRNA was used as an internal control. (B) Western blot analysis and quantification of phospho-Stat3/total Stat3 and phospho-p38/total p38 proteins in the liver of WT or *Pgrmc1* KO mice in feeding status. Hsp90 was used for an internal control. Values represent means ± S.D. * $p<0.05$. ** $p<0.01$. Total RNA was isolated from the liver of WT or *Pgrmc1* KO mice (n = 4). The protein analysis was repeated at least three times. WT, wild-type; KO, knockout.

each group (Fig. 3D). These results suggest that Pgrmc1 is not related to the hepcidin-glucose or hepcidin-insulin responses.

Cyclic AMP activator increased hepatic *Hamp* mRNA expression through high Pgrmc1 levels

As phosphorylation of cAMP-response element-binding protein (Creb) increases *Hamp* transcription [16] and Pgrmc1 increases cAMP activation during fasting [17], it is possible that Pgrmc1 controls *Hamp* mRNA transcription via cAMP activation. To determine whether the cAMP activator increased *Hamp* mRNA levels, we treated HepG2 cells with forskolin, which is a substitute for glucagon. CREB was phosphorylated by forskolin treatment. As the forskolin concentration increased in a dose-dependent manner, *HAMP* mRNA levels also significantly increased (Fig. 4A and B). When *PGRMC1* was knocked down by si*PGRMC1* in HepG2 cells, *HAMP* mRNA showed low reactivity against forskolin compared to the negative control

https://doi.org/10.12729/jbtr.2023.24.4.117

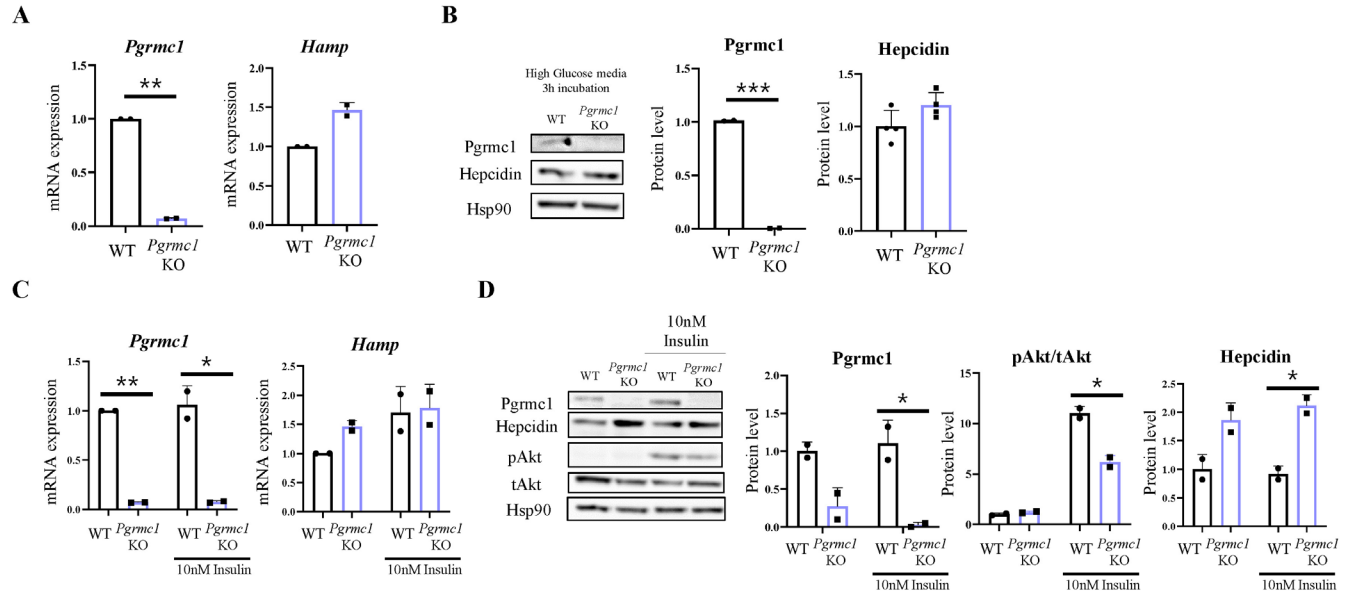

Fig. 3. Hepcidin expression in primary hepatocyte of *Pgrmc1* KO mice with insulin or glucose treatment. (A) *Pgrmc1* and *Hamp* mRNA expression in primary hepatocyte of WT or *Pgrmc1* KO. The hepatocytes were incubated with DMEM-high glucose medium including 4,500 mg/dL glucose for 3 hr. *Rplp0* mRNA was used as an internal control. Values represent means ± S.D. ** $p<0.01$. (B) Western blot analysis and quantification of Pgrmc1 and Hepcidin proteins in primary hepatocyte of WT or *Pgrmc1* KO. Hsp90 was used for internal control. Values represent means ± S.D. *** $p<0.001$. (C) *Pgrmc1* and *Hamp* mRNA expression in primary hepatocyte of WT or *Pgrmc1* KO. The hepatocytes were treated with 10 nM Insulin for 3 hr after incubated with DMEM-high glucose medium including 4,500 mg/dL glucose without FBS for 3 hr. *Rplp0* mRNA was used as an internal control. Values represent means ± S.D. * $p<0.05$. ** $p<0.01$. (D) Western blot analysis and quantification of Pgrmc1, Hepcidin and phospho-Akt/total Akt proteins in primary hepatocyte of WT or *Pgrmc1* KO. Hsp90 was used for internal control. Values represent means ± S.D. * $p<0.05$. All primary cell experiments (n = 3) were performed technical repeat for twice. WT, wild-type; KO, knockout; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum.

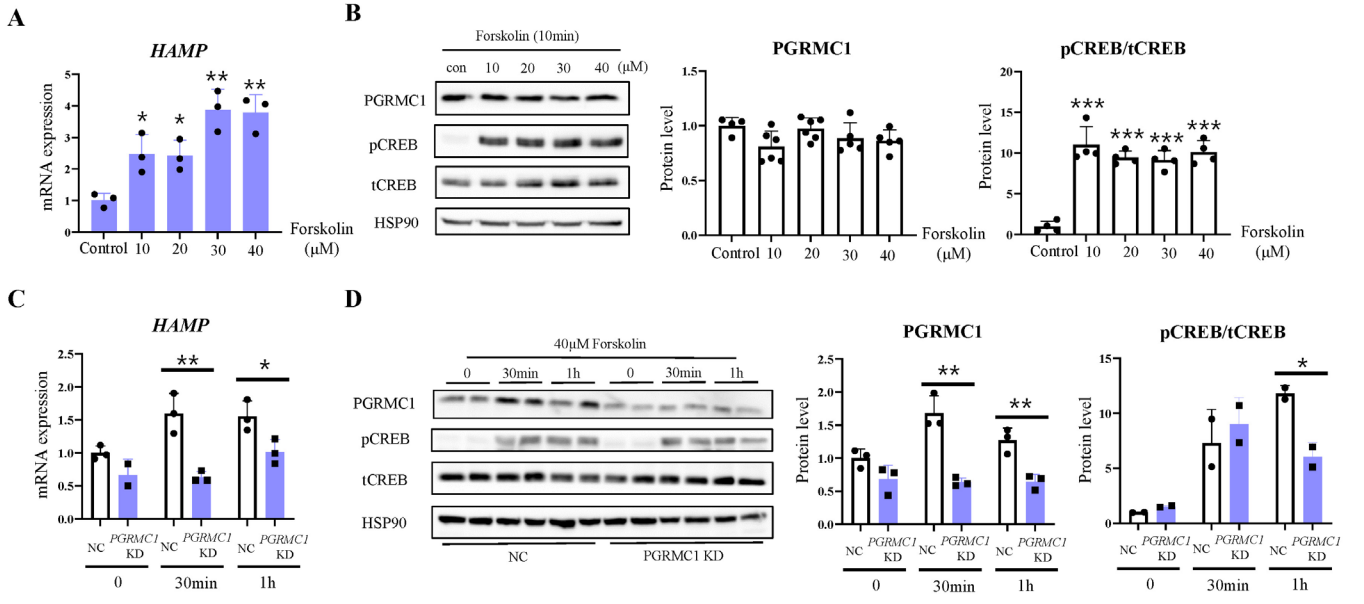

Fig. 4. Forskolin treatment increased *HAMP* mRNA, but *PGRMC1* knockdown did not increase in HepG2 cells. (A) *HAMP* mRNA expression in HepG2 cells with forskolin treatment (0, 10, 20, 30, 40 μM) for 10 min. *RPLP0* mRNA was used as an internal control. Values represent means ± S.D. * $p<0.05$. ** $p<0.01$. (B) Western blot analysis and quantification of PGRMC1 and phospho-CREB/total CREB proteins in HepG2 cells with forskolin treatment. HSP90 was used for an internal control. Values represent means ± S.D. *** $p<0.001$. (C) *HAMP* mRNA expression. HepG2 cells transfected with Negative control siRNA or *PGRMC1* siRNA and treated with 40μM forskolin for 30 min or 1 hr. *RPLP0* mRNA was used as an internal control. Values represent means ± S.D. * $p<0.05$. ** $p<0.01$. (D) Western blot analysis and quantification of PGRMC1 and phospho-CREB/total CREB proteins in HepG2 cells. HSP90 was used for internal control. Values represent means ± S.D. * $p<0.05$. ** $p<0.01$. All cell experiments (n = 3) were repeated at least three times.

(NC), suggesting that PGRMC1 promotes *HAMP* mRNA transcription via cAMP activation (Fig. 4C and D).

DISCUSSION

Hepcidin is a critical limiting factor for iron absorption. Iron deficiency anemia is the most prevalent nutritional disorder worldwide, and its risk is higher among women who experience periods that are especially long or that include very heavy bleeding [3]. Women have lower iron levels than men, and serum pro-hepcidin levels are significantly higher in men than in healthy women [18]. However, girls show higher serum hepcidin levels than boys [19]. These reports suggest that serum hepcidin levels may be affected by iron depletion, including ongoing blood loss, recent pregnancy, and inadequate dietary iron intake. Notably, female laboratory rats that did not experience menstrual bleeding showed higher hepatic hepcidin levels than male rats [20]. Chronic low iron concentrations may affect hepcidin levels more than other factors. Therefore, clarification of the mechanism of hepcidin regulation using physiological approaches is necessary to resolve the instability of iron homeostasis.

Some studies have shown that hepcidin regulates iron homeostasis and that various factors regulate its expression [21]. Hepcidin is known to be regulated via the BMP/HJV/SMAD pathway and JAK or IL-6/STAT3 signaling [15, 22, 23]. Moreover, glucose and insulin directly or indirectly upregulate hepcidin [17, 24]. Therefore, iron overload is a common symptom of type 2 diabetes involving insulin secretion disorders [25]. Pgrmc1 regulates hepcidin levels in HepG2 cells and zebrafish [14]. In the present study, we confirmed that *Hamp* mRNA expression and hepcidin protein levels were decreased in *Pgrmc1* KO mice and suggest that Pgrmc1 regulates hepcidin in mammals. Many factors, such as low intercellular and extracellular iron concentrations, inflammation, hypoxia, and erythropoiesis, promote *Hamp* mRNA transcription. Thus, we investigated the relationship between these factors and Pgrmc1; however, we could not determine the mechanism by which Pgrmc1 is directly regulated in the mouse liver. *Hamp* mRNA and hepcidin protein levels were significantly different between WT and *Pgrmc1* KO mice. This finding suggests that the internal environment of *Pgrmc1* KO mice has low hepcidin levels.

Pgrmc1 plays important roles in the body, including insulin secretion, cAMP activation, and hormone synthesis [26, 27]. *Pgrmc1* KO mice show slower glucose uptake into the intercellular space than WT mice because of low insulin secretion. This finding suggests that insulin affects the hepcidin levels in *Pgrmc1* KO mice. Adenine dietary supplementation in mice leads to increased hepatic hepcidin expression via the BMP/SMAD pathway. Therefore, adenine-mediated cAMP activation increases hepcidin levels [28]. Notably, when cAMP-mediated Creb increases *Hamp* mRNA transcription [16], Pgrmc1 increases cAMP levels under limited or impaired insulin action [17]. Thus, *Pgrmc1* KO mice can potentially have low insulin levels and low cAMP activation. Therefore, these conditions can be used to produce *Pgrmc1* KO mice with low hepcidin levels.

Our results described a possible beneficial effect of Pgrmc1 on iron metabolism. Although

https://doi.org/10.12729/jbtr.2023.24.4.117

the direct linking between Pgrmc1 and hepcidin was not recognized *in vitro*, loss of Pgrmc1 significantly decreased the hepatic hepcidin level. Under the condition of cAMP activation, PGRMC1 induces *HAMP* mRNA transcription. Considering these results, the internal environment caused by the lack of Pgrmc1 may be a critical factor in anemia.

REFERENCES

1. Pagani A, Nai A, Silvestri L, Camaschella C. Hepcidin and anemia: a tight relationship. Front Physiol 2019;10:1294.
2. Savarese G, von Haehling S, Butler J, Cleland JGF, Ponikowski P, Anker SD. Iron deficiency and cardiovascular disease. Eur Heart J 2023;44:14-27.
3. Safiri S, Kolahi AA, Noori M, Nejadghaderi SA, Karamzad N, Bragazzi NL, Sullman MJM, Abdollahi M, Collins GS, Kaufman JS, Grieger JA. Burden of anemia and its underlying causes in 204 countries and territories, 1990–2019: results from the Global Burden of Disease Study 2019. J Hematol Oncol 2021;14:185.
4. Walter PB, Knutson MD, Paler-Martinez A, Lee S, Xu Y, Viteri FE, Ames BN. Iron deficiency and iron excess damage mitochondria and mitochondrial DNA in rats. Proc Natl Acad Sci USA 2002;99:2264-2269.
5. Ginzburg Y, An X, Rivella S, Goldfarb A. Normal and dysregulated crosstalk between iron metabolism and erythropoiesis. eLife 2023;12:e90189.
6. Yanatori I, Kishi F. DMT1 and iron transport. Free Radic Biol Med 2019;133:55-63.
7. Scheers N. Regulatory effects of Cu, Zn, and Ca on Fe absorption: the intricate play between nutrient transporters. Nutrients 2013;5:957-970.
8. Petrak J, Vyoral D. Hephaestin: a ferroxidase of cellular iron export. Int J Biochem Cell Biol 2005;37:1173-1178.
9. Billesbølle CB, Azumaya CM, Kretsch RC, Powers AS, Gonen S, Schneider S, Arvedson T, Dror RO, Cheng Y, Manglik A. Structure of hepcidin-bound ferroportin reveals iron homeostatic mechanisms. Nature 2020;586:807-811.
10. Nemeth E, Ganz T. The role of hepcidin in iron metabolism. Acta Haematol 2009;122:78-86.
11. Cahill MA, Jazayeri JA, Catalano SM, Toyokuni S, Kovacevic Z, Richardson DR. The emerging role of progesterone receptor membrane component 1 (PGRMC1) in cancer biology. Biochim Biophys Acta 2016;1866:339-349.
12. McGuire MR, Mukhopadhyay D, Myers SL, Mosher EP, Brookheart RT, Kammers K, Sehgal A, Selen ES, Wolfgang MJ, Bumpus NN. Progesterone receptor membrane component 1 (PGRMC1) binds and stabilizes cytochromes P450 through a heme-independent mechanism. J Biol Chem 2021;297:101316.
13. Chen YJ, Knupp J, Arunagiri A, Haataja L, Arvan P, Tsai B. PGRMC1 acts as a size-selective cargo receptor to drive ER-phagic clearance of mutant prohormones. Nat Commun 2021;12:5991.
14. Li X, Rhee DK, Malhotra R, Mayeur C, Hurst LA, Ager E, Shelton G, Kramer Y, McCulloh D, Keefe D, Bloch KD, Bloch DB, Peterson RT. Progesterone receptor membrane component-1

regulates hepcidin biosynthesis. J Clin Invest 2016;126:389-401.
15. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. Blood 2006;108:3204-3209.
16. Wang C, Fang Z, Zhu Z, Liu J, Chen H. Reciprocal regulation between hepcidin and erythropoiesis and its therapeutic application in erythroid disorders. Exp Hematol 2017;52:24-31.
17. Lee SR, Choi WY, Heo JH, Huh J, Kim G, Lee KP, Kwun HJ, Shin HJ, Baek IJ, Hong EJ. Progesterone increases blood glucose via hepatic progesterone receptor membrane component 1 under limited or impaired action of insulin. Sci Rep 2020;10:16316.
18. Jasiniewska J, Zekanowska E, Dymek G, Gruszka M. The impact of sex and age on serum prohepcidin concentration in healthy adults. EJIFCC 2009;20:129-135.
19. Jaeggi T, Moretti D, Kvalsvig J, Holding PA, Tjalsma H, Kortman GAM, Joosten I, Mwangi A, Zimmermann MB. Iron status and systemic inflammation, but not gut inflammation, strongly predict gender-specific concentrations of serum hepcidin in infants in rural Kenya. PLOS ONE 2013;8:e57513.
20. Kong WN, Niu QM, Ge L, Zhang N, Yan SF, Chen WB, Chang YZ, Zhao S. Sex differences in iron status and hepcidin expression in rats. Biol Trace Elem Res 2014;160:258-267.
21. Rishi G, Wallace DF, Subramaniam VN. Hepcidin: regulation of the master iron regulator. Biosci Rep 2015;35:e00192.
22. Silvestri L, Nai A, Dulja A, Pagani A. Hepcidin and the BMP-SMAD pathway: an unexpected liaison. Vitam Horm 2019;110:71-99.
23. Wang CY, Babitt JL. Hepcidin regulation in the anemia of inflammation. Curr Opin Hematol 2016;23:189-197.
24. Aigner E, Felder TK, Oberkofler H, Hahne P, Auer S, Soyal S, Stadlmayr A, Schwenoha K, Pirich C, Hengster P, Datz C, Patsch W. Glucose acts as a regulator of serum iron by increasing serum hepcidin concentrations. J Nutr Biochem 2013;24:112-117.
25. Wang H, Li H, Jiang X, Shi W, Shen Z, Li M. Hepcidin is directly regulated by insulin and plays an important role in iron overload in streptozotocin-induced diabetic rats. Diabetes 2014;63:1506-1518.
26. Zhang M, Robitaille M, Showalter AD, Huang X, Liu Y, Bhattacharjee A, Willard FS, Han J, Froese S, Wei L, Gaisano HY, Angers S, Sloop KW, Dai FF, Wheeler MB. Progesterone receptor membrane component 1 is a functional part of the glucagon-like peptide-1 (GLP-1) receptor complex in pancreatic β cells. Mol Cell Proteomics 2014;13:3049-3062.
27. Rohe HJ, Ahmed IS, Twist KE, Craven RJ. PGRMC1 (progesterone receptor membrane component 1): a targetable protein with multiple functions in steroid signaling, P450 activation and drug binding. Pharmacol Ther 2009;121:14-19.
28. Zhang Y, Wang X, Wu Q, Wang H, Zhao L, Wang X, Mu M, Xie E, He X, Shao D, Shang Y, Lai Y, Ginzburg Y, Min J, Wang F. Adenine alleviates iron overload by cAMP/PKA mediated hepatic hepcidin in mice. J Cell Physiol 2018;233:7268-7278.

https://doi.org/10.12729/jbtr.2023.24.4.117