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PAQR3 modulates blood cholesterol level by facilitating interaction between LDLR and PCSK9

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ABSTRACT

Objective: Low-density lipoprotein cholesterol (LDL-C) is the hallmark of atherosclerotic cardiovascular diseases. The hepatic LDL receptor (LDLR) plays an important role in clearance of circulating LDL-C. PCSK9 facilitates degradation of LDLR in the lysosome and antagonizing PCSK9 has been successfully used in the clinic to reduce blood LDL-C level. Here we identify a new player that modulates LDLR interaction with PCSK9, thus controlling LDLR degradation and cholesterol homeostasis.
Methods: The blood LDL-C and cholesterol levels were analyzed in mice with hepatic deletion of *Paqr3* gene. The half-life of LDLR was analyzed in HepG2 cells. The interaction of PAQR3 with LDLR and PCSK9 was analyzed by co-immunoprecipitation and immunofluorescent staining.
Results: The blood LDL-C and total cholesterol levels in the mice with hepatic deletion of *Paqr3* gene were significantly lower than the control mice after feeding with high-fat diet ($p < 0.001$ and $p < 0.05$ respectively). The steady-state level of LDLR protein is elevated by *Paqr3* knockdown/deletion and reduced by PAQR3 overexpression. The half-life of LDLR protein is increased by *Paqr3* knockdown and accelerated by PAQR3 overexpression. PAQR3 interacts with the β-sheet domain of LDLR and the P-domain of PCSK9 respectively. In addition, PAQR3 can be localized in early endosomes and colocalized with LDLR, PCSK9 and LDL. Mechanistically, PAQR3 enhances the interaction between LDLR and PCSK9.
Conclusion: Our study reveals that PAQR3 plays a pivotal role in controlling hepatic LDLR degradation and blood LDL-C level via modulating LDLR-PCSK9 interaction.

1. Introduction

The plasma levels of cholesterol-carrying low-density lipoprotein (LDL-C) are closely associated with increased risk of atherosclerotic cardiovascular disease (ASCVD), one of the primary causes of mortality and morbidity in the world [1–3]. Decreasing plasma LDL-C level has been shown to reduce the risk of ASCVD and death [1]. The blood LDL-C level is mainly controlled by low-density lipoprotein receptors (LDLRs) in the liver to remove LDL-C from the blood [4,5]. Approximately 70% of circulating LDL-C is cleared via endocytosis through hepatic LDLRs [6–8]. Due to its pivotal role in cholesterol homeostasis, LDLR is regulated in hepatocytes through multiple signaling pathways [9]. Transcription of the LDLR gene is regulated by the sterol response element binding proteins (SREBPs) in a cholesterol-negative feedback pathway [1,9]. Post-translation regulation of LDLR is mainly mediated by proprotein convertase subtilisin/kexin type 9 (PCSK9), a member of the subtilisin serine protease family [10–13]. PCSK9 facilitates LDLR degradation by binding to LDLR and enhancing lysosome-mediated destruction of LDLR [14–16]. The importance of PCSK9 in modulating blood LDL-C level is best exemplified by the clinical success of using antibodies against PCSK9 to reduce LDL-C in healthy individuals as well as hypercholesterolemia patients [17–22].

LDLRs undergoes constant endocytosis via early endosome and cycles back to the plasma membrane, while the LDL-C cargo is degraded in the lysosome. In the presence of PCSK9, however, LDLRs take the route of lysosome-mediated degradation. PCSK9 binds to LDLR at the cell surface of hepatocytes in which the catalytic domain of PCSK9 interacts with the epidermal growth factor repeat A (EGF-A) domain of LDLR [23]. The LDLR-PCSK9 complex is internalized through clathrin-mediated endocytosis. Due to an additional electrostatic interaction at acidic pH between the C-terminal domain of PCSK9 and the ligand-binding domain of the LDLR, PCSK9 remains bound to LDLR in the

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sorting endosome [23,24]. As a consequence, LDLR fails to adopt a closed conformation and is degraded instead of being recycled back to the plasma membrane [23,24]. However, it is currently unknown whether LDLR-PCSK9 interaction is modulated by other molecules, a question to be addressed in this study.

The progestin and AdipoQ Receptor (PAQR) family is evolutionarily conserved and all the members within the family contain seven transmembrane domains [25]. This family has eleven members, i.e., from PAQR1 to PAQR11, and they possess different physiological functions. Within this family, PAQR1 and PAQR2 are receptors for adiponectin that plays an important role in maintaining insulin sensitivity [26]. PAQR4 was found to modulate ubiquitination of CDK4 and is implicated in tumorigenesis [27]. PAQR5 to PAQR8 are believed to serve as membrane receptors for progestin implicated in the regulation of reproduction [28]. PAQR10-11 were found to regulate Ras-mediated signaling by tethering Ras proteins to the Golgi apparatus [29]. The function of PAQR9 has not been reported. PAQR3 is mainly localized in the Golgi apparatus and functions primarily as a tumor suppressor via inhibiting pro-growth signaling pathways mediated by Raf kinases and PI3K/AKT [30–35]. PAQR3 can be redistributed into punctiform structures of autophagosome to control autophagy by integrating AMPK signaling or by modulating mTOR activity [36,37]. In this study, we identified that PAQR3 can be redistributed into early endosomes and plays a critical role in cholesterol homeostasis by affecting the stability of LDLR via modulation of LDLR-PCSK9 complex formation.

2. Methods

2.1. Materials and antibodies

The materials and antibodies used in this study were purchased as follows: anti-Flag tag from Sigma-Aldrich (F3165, Darmstadt, Germany); anti-GM130 from Abcam (ab52649, Cambridge, UK); anti-Tubulin from Sigma-Aldrich (T6199); anti-Myc tag from Santa Cruz Biotechnology (sc-40, Santa Cruz, CA); anti-GFP from Santa Cruz Biotechnology (sc-9996); anti-LDLR from Abcam (ab52818); anti-PCSK9 from Abcam (ab31762); Cycloheximide (CHX) from Sigma-Aldrich (C7698); EEA1 from BD Biosciences (610456, Franklin Lakes, New Jersy, USA); Alexa Fluor 546 goat anti-mouse IgG from Abcam (A11003); Alexa Fluor 546 goat anti-rabbit IgG from Abcam (A11035); Alexa Fluor 488 goat anti-mouse IgG from Abcam (A11029); Alexa Fluor 488 goat anti-rabbit IgG from Abcam (A11034); Alexa Fluor 633 goat anti-mouse IgG from Abcam (A21050).

2.2. Plasmids construction

The Myc-tagged PAQR3 was described as previously reported [30]. The Myc-tagged PCSK9 cDNA was purchased from SinoBiological.C-terminal Flag tag-fused PCSK9 was cloned in p3XFlag-CMV-10 vector (Sigma-Aldrich). GFP-PCSK9-P, GFP-PCSK9-CAT and GFP-PCSK9-CTD were generated by PCR using PCSK9 plasmid and cloned into pEGFP-C1 vector. Myc-tagged LDLR was purchased from SinoBiological (HG15813-CM, Beijing, China). The Flag-tagged LDLR was generated by PCR using the human LDLR plamid and cloned into p3XFlag-CMV-10 vector with confirmation by DNA sequencing.

2.3. Cell culture and transfection

HEK293T, Hela, and HepG2 cell lines were cultured in completed medium consisting of DMEM containing 10% FBS,100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C with 5% CO_2. Transient transfection was performed with the polyethylenimine (Sigma-Aldrich) method for HEK293T cells. Transfection in Hela cells was performed using PolyJet transfection reagents (Signagen, Maryland, USA). Establishment of HepG2 cells with stable overexpression of PAQR3 and knockdown of PAQR3 has been reported previously [38].

2.4. Co-immunoprecipitation, immunoblotting and confocal microscopy

HEK293T cells were washed with ice-cold PBS and then lysed with ice-cold lysis buffer (40 mM HEPES [pH 7.4], 120 mM NaCl, 1 mM EDTA, 10 mM pyrophosphate, 10 mM glycerophosphate, 0.3% CHAPS, and protease inhibitor cocktail) for 30 min at 4 °C. The homogenates were centrifuged for 15 min at 12,000 rpm at 4 °C. About 10% of the supernatant was harvested for western blotting analysis as inputs, while the remaining cell lysate was mixed with indicated primary antibodies

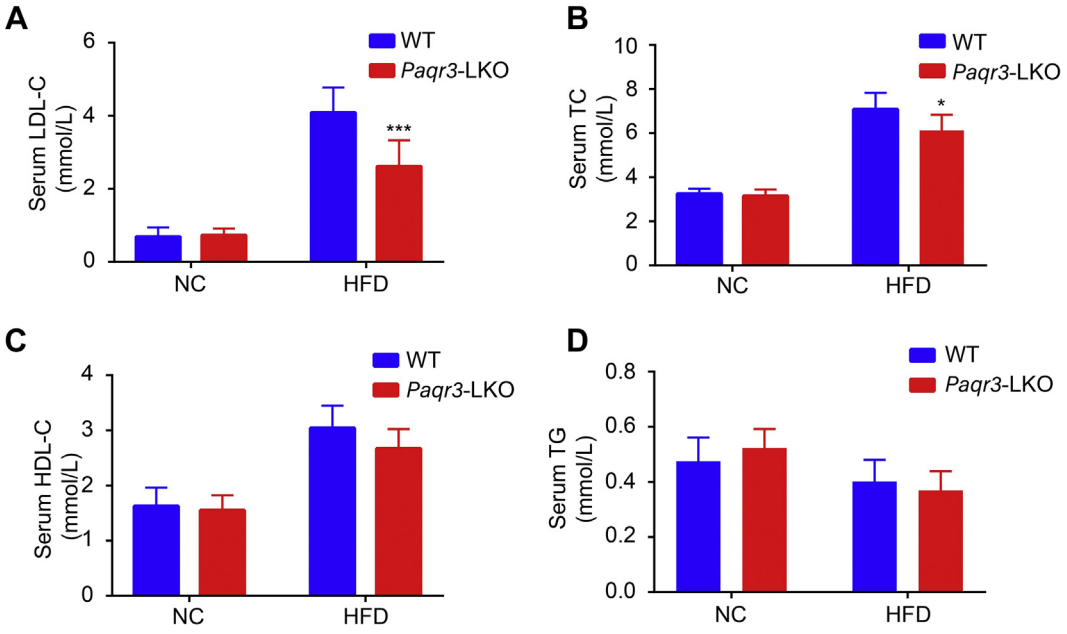

Fig. 1. Deletion of *Paqr3* in the liver decreases plasma LDL-C and cholesterol levels. (A, B) LDL-C level and total cholesterol levels in mice with liver-specific deletion of *Paqr3* (*Paqr3*-LKO) and the age-matched flox/flox littermates (WT) when fed with normal chow (NC) or high fat diet (HFD). Both the WT and *Paqr3*-LKO mice (male, 6 weeks old, n = 8 for each group) were fed with NC or HFD for 3 months. (C, D) Total triglyceride (TG) and HDL-C level in both WT mice and *Paqr3*-LKO mice fed with NC or HFD. The data are shown as means ± SD. * and *** represent $p < 0.05$ and $p < 0.001$ respectively between the WT and *Paqr3*-LKO mice.

with rotation overnight at 4 °C. 30 μl protein A/G plus agarose beads (Genescript, New Jersey, USA) were added for an additional 2 h at 4 °C. The immunoprecipitation beads were washed five times with lysis buffer, followed by Western blotting analysis. The methods for immunoblotting, immunofluorescence staining, and confocal analyses were described previously [30].

2.5. Live cell imaging

HepG2,Hela cells seeded on 35-mm culture dishes were incubated with 10 μg/ml Dil-LDL (YEASEN, Shanghai, China) at 37 °C for 10 min and then washed at medium 3 times. Cells were immediately imaged on a Zeiss (LSM880) confocal microscope equipped with a PlanApo 63× oil-immersion objective. Images were acquired at 5 s intervals for 10 min.

2.6. Animal studies

All animals were maintained and used in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Institute for Nutritional Sciences. All of the experimental procedures were carried out in accordance with the Chinese Academy of Sciences ethics commission with an approval number 2010-AN-8. Mice were maintained on a 12-h light/dark cycle at 25 °C. The liver-specific PAQR3-deleted mice were generated as previously described [38]. In short, PAQR3$^{tm1a(KOMP)Wtsi}$ (Strain ID) embryos with C57BL/6 background were ordered from KOMP Repository (Davis, CA, USA). The mice were bred with flp mice to release flox sites and then mated with mice expressing the Cre recombinase driven by the albumin promoter (Jackson Laboratory, Bar Harbor, ME, USA) to generate liver-specific *Paqr3* knockout mice (*Paqr3*-LKO).

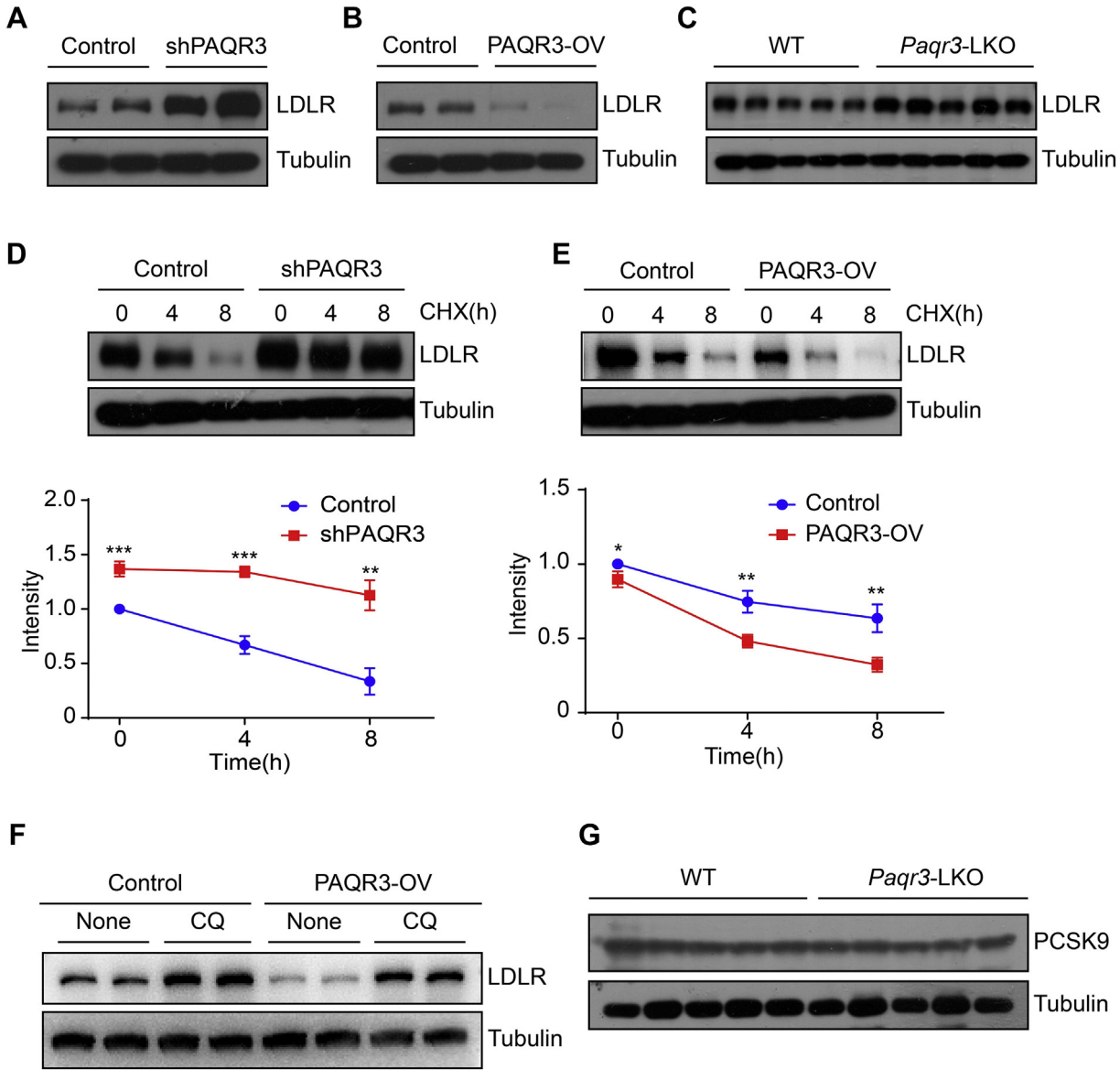

Fig. 2. PAQR3 affects the stability of LDLR protein. (A, B) PAQR3 modulates the LDLR protein level in HepG2 cells. HepG2 cells were infected with control lentivirus or the lentivirus containing PAQR3-specific shRNA (shPAQR3) (for A), or infected with control lentivirus or the lentivirus containing PAQR3 (PAQR3-OV) (for B). The cell lysate was used in immunoblotting with antibodies for LDLR and α-tubulin. (C) LDLR protein level is increased in the liver of *Paqr3*-deleted mice. The livers from wild type (WT) and liver-specific *Paqr3* knockout mice (*Paqr3*-LKO) were used in immunoblotting. (D, E) PAQR3 modulates the protein half life of LDLR in HepG2 cells. HepG2 cells as in A and B were treated with cycloheximide (CHX) for 0, 4, 8 h. The cell lysate was used in immunoblotting with the antibodies as indicated. The quantitative measurement of LDLR protein is shown in the lower panel (mean ± SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (F) Effect of lysosome inhibitor chloroquinone on LDLR protein level. HepG2 cells as in B were treated with or without chloroquinone (CQ) at 20 μM for 4 h. The cell lysate was used in immunoblotting. (G) *Paqr3* deletion has no effect on the protein level of PCSK9. Liver tissues from WT and *Paqr3*-LKO mice were used in immunoblotting to detect the protein level of PCSK9.

2.7. Statistical analysis

Student's *t*-test was used to analyse the data. p-Values < 0.5 was considered statistically significant and all results were shown as mean ± standard deviation (SD).

3. Results

3.1. Deletion of Paqr3 gene in the liver decreases plasma cholesterol level

To investigate the potential functions of PAQR3 in the liver, we generated a liver-specific *Paqr3* knockout mouse model (*Paqr3*-LKO) using Cre-loxP system [38]. We analyzed the blood lipid profile of the mice fed with high fat diet for 3 months. The plasma levels of both LDL-C and total cholesterol (TC) were significantly reduced by deletion of *Paqr3* gene (Fig. 1A and B, p = 0.0004 and p = 0.02 respectively). However, Paqr3 deletion in the liver had little effect on the plasma HDL-C and triglyceride (TG) levels (Fig. 1C and D). Therefore, these data indicate that hepatic PAQR3 has a functional role in regulating cholesterol level in the blood.

In addition, deletion of *Paqr3* in the liver led to slight reduction of HFD-induced obesity, without changes of body weight under normal chow (Supplemental Fig. 1). Deletion of *Paqr3* in the liver also significantly reduced the tryglyceride accumulation in the liver and fatty liver formation under HFD condition (Supplmental Fig. 2). These results suggested that PAQR3 in the liver is involved in the regulation of energy metabolism and hepatic steatosis when fed with HFD.

3.2. PAQR3 affects stability of LDLR protein

As LDLRs in hepatocytes control the transfer of LDL-C into the cells and determines the blood cholesterol level, we investigated whether PAQR3

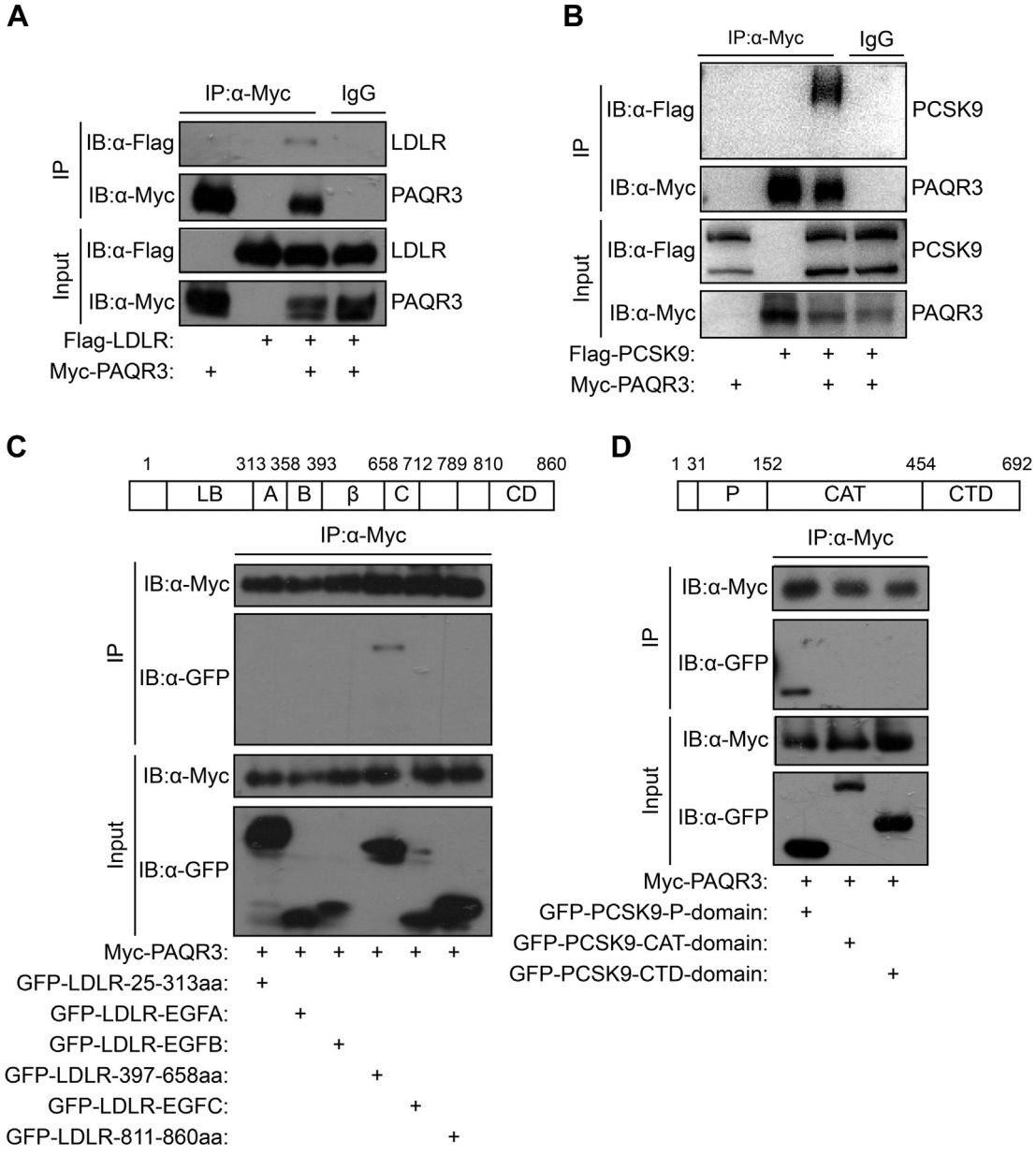

Fig. 3. PAQR3 interacts with LDLR and PCSK9. (A) Interaction between PAQR3 and LDLR. Myc-tagged PAQR3 and Flag-tagged LDLR were co-expressed in HEK293T cells. After transfection for 24 h, the cell lysate was used in immunoprecipitation (IP) and immunoblotting (IB) with the antibodies as indicated. (B) Interaction between PAQR3 and PCSK9. Myc-tagged PAQR3 and Flag-tagged PCSK9 were co-expressed in HEK293T cells. After transfection for 24 h, the cell lysate was used in IP and IB. (C) PAQR3 interacts with the β sheet domain of LDLR. PAQR3 and different domain of LDLR were expressed in HEK293T cells and the cell lysate was used in IP and IB. (D) PAQR3 interacts with the P domain of PCSK9. PAQR3 and different domain of PCSK9 were expressed in HEK293T cells and the cell lysate was used in IP and IB.

was able to alter the protein level of LDLR. When PAQR3 was knocked down in HepG2 cells, the basal level of LDLR protein was markedly elevated (Fig. 2A). In contrast, overexpression of PAQR3 in HepG2 cells reduced the protein level of LDLR (Fig. 2B). Consistently, the LDLR protein level in the mouse liver was also increased by *Paqr3* deletion in the liver (Fig. 2C). We next analyzed the half-life of LDLR protein in HepG2 cells by treating cells with cycloheximide to stop synthesis of new proteins. Knockdown of PAQR3 apparently slowed down the degradation rate of LDLR protein (Fig. 2D). In contrast, overexpression of PAQR3 accelerated the protein degradation rate of LDLR (Fig. 2E). Taken together, these data demonstrate that PAQR3 can reduce the stability of LDLR protein by increasing its degradation. Furthermore, treatment of the HepG2 cells with chloroquine, an inhibitor of lysosome function, was able to accumulate similar level of LDLR protein in both control and PAQR3-overexpressed cells (Fig. 2F), indicating that the accelerated degradation of LDLR in the presence of overexpressed PAQR3 is still mediated by the lysosome pathway.

3.3. PAQR3 interacts with LDLR and PCSK9

We next explored whether or not PAQR3 affects the function of PCSK9 to modulate LDLR degradation. In theory, PAQR3 might affect PCSK9-mediated LDLR degradation by two possible mechanisms: altering PCSK9 expression/maturation or affecting PCSK9/LDLR interaction. In *Paqr3*-deleted liver, the protein level of the mature form of PCSK9 was not altered (Fig. 2G), thus ruling out the first possibility. We therefore focused on the potential function of PAQR3 on PCSK9-LDLR interaction. Both Myc-tagged PAQR3 and Flag-tagged LDLR were expressed in HEK293T cells. Immunoprecipitation of the cell lysate with an anti-Myc antibody was able to detect the Flag-tagged LDLR (Fig. 3A), indicating that PAQR3 could interact with LDLR. Similarly, a co-immunoprecipitation assay revealed that PAQR3 could also interact with PCSK9 (Fig. 3B). We next analyzed the potential domains of LDLR and PCSK9 involved in the interaction with PAQR3. We cloned different domains of LDLR and PCSK9 respectively. For LDLR, we cloned the ligand binding domain (25-313 aa), the three EGF domains (317-353 aa for EGFA, 354-393 aa for EGFB and 666-712 aa for EGFC domains respectively), the β-sheet domain (397-658 aa), and the C-terminal domain (810 to 860 aa) (Fig. 3C, top panel). For PCSK9, we cloned the P domain (31-152 aa), the catalytic domain (153-454 aa) and the C-terminal domain (455-692 aa) (Fig. 3D, top panel). By co-immunoprecipitation assays, we found that PAQR3 only interacted with the β-sheet of LDLR and the P-domain of PCSK9 respectively (Fig. 4C and D). These data, therefore, indicate that PAQR3 can interact with both LDLR and PCSK9 via specific domains.

3.4. PAQR3 can be localized in early endosome and colocalized with LDLR, PCSK9 and LDL

As previously reported [30], PAQR3 is mainly a Golgi-localized membrane protein (Fig. 4A). However, careful examination of PAQR3 distribution demonstrated that some PAQR3 proteins could be also distributed in punctate structures (Fig. 4B). Interestingly, these PAQR3-containing punctate structures were clearly co-localized with EEA1 (Fig. 4B), an

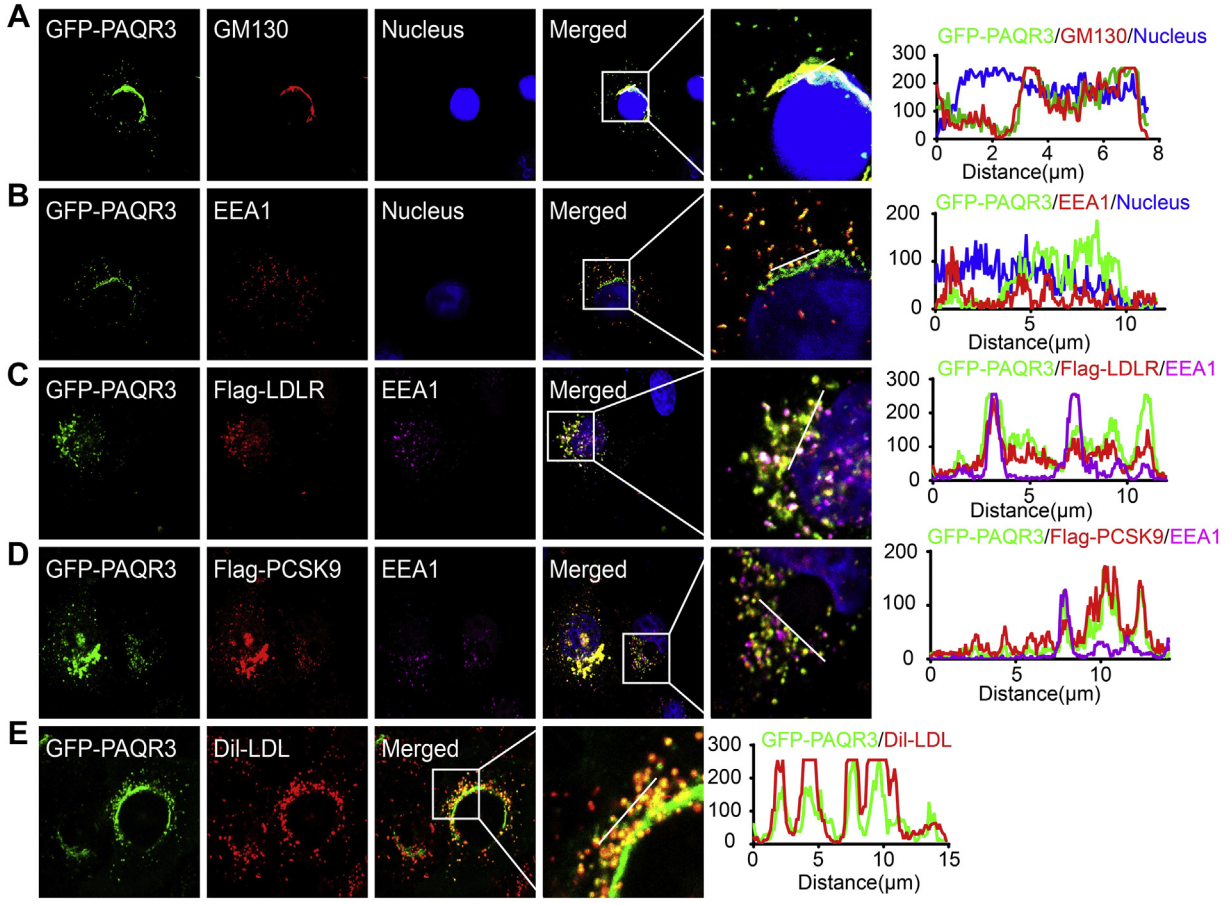

Fig. 4. PAQR3 can be localized in early endosome, co-localized with LDLR and PCSK9. (A–D) Localization studies of PAQR3 in Hela cells. Hela cells were transiently transfected with GFP-fused PAQR3 alone (for A and B), GFP-fused PAQR3 with Flag-tagged LDLR (for C), GFP-fused PAQR3 with Flag-tagged PCSK9 (for D). The cells were used in immunofluorescent staining. The Golgi was stained with an antibody against GM130. The nucleus was stained with Hoechst 33342. The early endosomes was stained with antibody against EEA1. LDLR and PCSK9 were stained with an anti-Flag antibody. (E) PAQR3 colocalizes with Dil-LDL. HepG2 cells seeded on 35-mm culture dishes were incubated with 10 μg/ml Dil-LDL at 37 °C for 10 min, and then washed with culture medium for 3 times. The cells were immediately imaged on a Zeiss (LSM880) confocal microscope equipped with a PlanApo 63× oil-immersion objective. Intensity plots of signal intensity (y axis) against distance in μm (x axis) are used to indicate occurrence of overlaps between the red and green channels (shown on the right panels).

early endosome marker. Furthermore, the PAQR3-containing punctiform structures had obvious co-localization with LDLR and PCSK9 (Fig. 4C and D). In addition, live cell imaging assay revealed that the punctuate PAQR3 was partly co-localized with Dil-LDL (Fig. 4E). Collectively, these data suggest that PAQR3 can be localized in early endosomes where PAQR3 can be colocalized with LDLR, PCSK9 and LDL.

3.5. PAQR3 enhances the interaction between LDLR and PCSK9

The degradation of LDLR protein in sorting endosome/lysosome is enhanced by PCSK9 binding [23,24]. As we observed that PAQR3 was able to accelerate LDLR degradation and interact with both LDLR and PCSK9, we speculated that PAQR3 might enhance LDLR degradation by enhancing

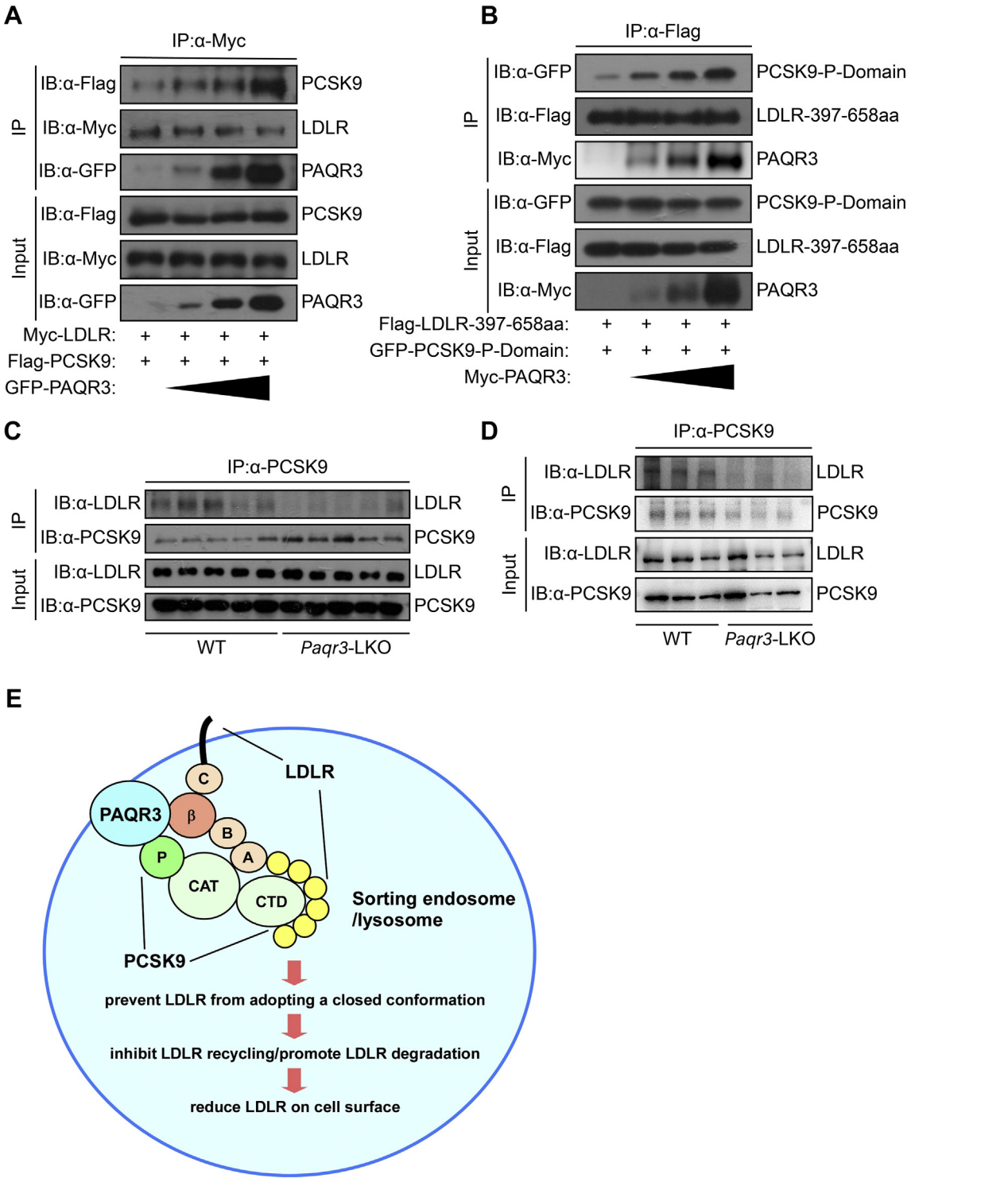

Fig. 5. PAQR3 enhances the interaction between LDLR and PCSK9. (A) PAQR3 enhances the interaction between LDLR and PCSK9. HEK293T cells were transfected the plasmids as indicated, followed by immunoprecipitation (IP) and immunoblotting (IB) with the antibodies as indicated. (B) PAQR3 dose-dependently enhanced the interaction between the β-sheet of LDLR and the P-domain of PCSK9. HEK293T cells were transfected the plasmids as indicated, followed by IP and IB with the antibodies as indicated. (C) The interaction between LDLR and PCSK9 were reduced in the liver of *Paqr3*-deleted mice. The livers from wild type (WT) and liver-specific *Paqr3* knockout mice (*Paqr3*-LKO) were used in IP and IB using the antibodies as indicated. (D) The interaction between LDLR and PCSK9 were reduced by *Paqr3* deletion in primary hepatocytes. The primary hepatocytes from WT and *Paqr3*-LKO mice were used in IP and IB. (E) A model to depict the function of PAQR3 to enhance LDLR-PCSK9 interaction, thereby modulating LDLR degradation in the lysosome.

the LDLR-PCSK9 interaction. Using a co-immunoprecipitation assay, we found that overexpression of PAQR3 was able to dose-dependently enhance the interaction of LDLR with PCSK9 (Fig. 5A). In line with this observation, PAQR3 dose-dependently enhanced the interaction between the β-sheet of LDLR and the P-domain of PCSK9 (Fig. 5B). In contrast, the interaction of endogenous PCSK9 with endogenous LDLR was reduced by *Paqr3* deletion in the liver tissue (Fig. 5C). Likewise, the interaction of endogenous PCSK9 with endogenous LDLR was reduced by *Paqr3* deletion in primary hepatocytes isolated from the mice (Fig. 5D). These data, collectively, indicate that PAQR3 modulates LDLR degradation via enhancing the interaction of LDLR with PCSK9 (Fig. 5).

4. Discussion

Our series of studies at both the cellular and animal levels illustrated that PAQR3 is able to modulate the protein level of LDLR and alter the plasma LDL-C level. Mechanistically, PAQR3 enhances the interaction of the LDLR with PCSK9 via binding to the β-sheet domain of LDLR and the P-domain of PCSK9 (Fig. 5E). Therefore, there are three interfaces that facilitate LDLR-PCSK9 interaction (i.e., the interaction of the EGF-A domain of LDLR with the catalytic domain of PCSK9, the interaction of the ligand binding domains of LDLR with the C-terminal domain of PCSK9 under acidic condition, and the PAQR3-mediated interaction of the the β-sheet domain of LDLR with the P-domain of PCSK9 (Fig. 5E)). The combination of these three interaction forces would favor LDLR to maintain a closed confirmation in sorting endosome, thereby aiding in degradation of LDLR in the lysosome. The activity of PAQR3 to facilitate LDLR-PCSK9 interaction appears to play a critical role in vivo as deletion of *Paqr3* leads to reduced LDLR-PCSK9 complex formation and increased steady state LDLR protein level in the liver, leading to a decline of blood LDL-C and cholesterol level. It is noteworthy here, however, that change or polymorphism of *Paqr3* gene has not been reported to be associated with LDL-C level in humans. It will be important to explore this issue in human subjects in the future.

PAQR3 can modulate cholesterol homeostasis via multiple pathways. Previously, it was found that PAQR3 is able to facilitate trafficking of Scap/SREBP2 complex from ER to the Golgi, thus enhancing SREBP2 activation in the Golgi and increasing cholesterol biosynthesis [39]. In this study, we discovered that PAQR3 enhances LDLR-PCSK9 interaction to decrease the LDLR on the the surface of hepatocytes. Both of the effects of PAQR3 would result in an increase in the blood cholesterol level. Considering the ability of PAQR3 to control cholesterol homeostasis, it would be of great importance to explore such function of PAQR3 in the future as a strategy to control blood cholesterol level. For example, using small chemicals to block PAQR3 interaction with LDLR and/or PCSK9 would favor dissociation of PCSK9 from LDLR, leading to increased recycling to LDLR to the plasma membrane of hepatocytes and consequently result in a decrease in blood LDL-C level.

One unanswered question in this study is how PAQR3 can facilitate the interaction of LDLR with PCSK9 at the subcellular level. PAQR3 is mainly localized in the Golgi apparatus as suggested by previous studies [30,40]. Careful examination revealed that PAQR3 can also be localized in the early endosomes (Fig. 4). Topology analysis of PAQR3 demonstrated that its N-terminus faces the cytoplasmic side of the Golgi apparatus, while its first, third, and fiftieth loop between the transmembrane domains, as well as the C-terminus, face the lumen [40]. Based on the configuration of topology, only the domains facing the lumen would be involved in the interaction with LDLR-PCSK9 within endosomes as both of the proteins are localized in the lumen side of the sorting endosome after being endocytosized [23,24]. Our hypothesis is that PAQR3-containing early endosomes can fuse with the endocytosed LDLR/PCSK9-containing vesicles, thereby facilitating LDLR-PCSK9 interaction and favoring LDLR degradation in the lysosomes. If this were the case, blockding the fusion of the PAQR3-containing vesicles with the LDLR/PCSK9-containing vesicles can be explored as a novel strategy to lower blood cholesterol level.

Author contribution

Y.C. conceptualized the study. M.H. and Y.C. designed the study and wrote the paper. M.H. and Z.Z. performed the experiments. Q.C., X.Y., S.W., J.Z., and M.B. provided technical assistance.

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Conflict of interest

All the authors declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2019.02.005.

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