Genes & Diseases (2022) 9, 1397–1400

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

RAPID COMMUNICATION

# Sphingomyelin phosphodiesterase acid like 3B (SMPDL3b) regulates Perilipin5 (PLIN5) expression and mediates lipid droplet formation

Lipid droplets (LDs) are lipid storage organelles with a central hydrophobic core of neutral lipids that is enclosed in a monolayer of phospholipids. Though the storage of lipids, mainly cholesterol and triglycerides, was thought to be the primary function of LDs, studies have shown that they have important functions in maintaining lipid homeostasis and modulating various signaling pathways. Abnormal lipid accumulation has been recognized as a key feature in several human diseases such as obesity, diabetes, cancer, neurological diseases, cardiovascular diseases, insulin resistance, liver diseases and kidney diseases. LDs play an important role in innate immunity, in the fight against infections, and in the replication of SARS-CoV-2. Hence a tight regulation of LD metabolism is essential for proper functioning of a cell. We previously reported an important role for LD accumulation and the progression of kidney diseases,[1] including diabetic kidney disease and focal segmental glomerulosclerosis (FSGS). In FSGS, we found that glomerular LD accumulation coincided with decreased expression of sphingomyelinase phosphodiesterase like 3b (SMPDL3b), an enzyme we showed to regulate ceramide-1-phosphate levels in podocytes,[2,3] suggesting a role for SMPDL3b in sphingolipid metabolism. However, if SMPDL3b regulates LD homeostasis is unknown. The present study was aimed at determining if SMPDL3b regulates LD homeostasis in immortalized human podocytes, which are terminally differentiated cells that have a key role in the glomerular filtration barrier. We found that SMPDL3b is localized to LDs and that reduced expression of SMPDL3b is associated with an increase in the number of LDs, increased levels of triglycerides (TAG) and cholesterol esters, increased uptake of fatty acids and decreased lipolysis. Reduced expression of SMPDL3b leads to an increase in PLIN5, heat shock cognate protein (HSC70) and lysosome-associated membrane protein 2A (LAMP2A) expression. Selonsertib treatment corrected all the phenotypes observed in immortalized human podocytes with reduced SMPDL3b expression.

To investigate the role of SMPDL3b in LD homeostasis, LD fractions were isolated from immortalized human podocytes with decreased SMPDL3b expression (siSMPDL3b) and of control podocytes (Ctrl). Western blot analysis of isolated LD fractions revealed SMPDL3b protein expression in LD, and demonstrated decreased expression in siSMPDL3b podocytes when compared to Ctrl podocytes (Fig. 1A, B). Interestingly, reduced presence of SMPDL3b in LDs was associated with an increase in the number (Fig. 1C) and intensity (Fig. 1D) of LDs as well as of cellular triglyceride (TAG) (Fig. 1E) and cholesterol ester levels (Fig. 1F), suggesting an important role for SMPDL3b in LD homeostasis and the lipid composition of LDs in human podocytes.

It has been demonstrated that SMPDL3b alters the phospholipid composition of cellular membranes of macrophages and of sphingolipids in podocytes. Phosphatidylcholine (PC) is the most abundant phospholipid followed by phosphatidylethanolamine (PE) and phosphatidylserine (PS) on the surface of LDs. We therefore determined the phospholipid composition of LDs and found an increase in all three phospholipid species of PC, PE, PS, as well as of lysophosphatidylcholine (LPC) in LDs isolated from siSMPDL3b human podocytes (Fig. S2). This increase in the amount of phospholipids is likely due to the increase in the number of LDs in siSMPDL3b human podocytes.

The accumulation of LDs can be caused by excess fatty acids that are esterified and stored in LDs or by defective lipolysis. To investigate the mechanism by which SMPDL3b deficiency contributes to LD accumulation, levels of FA

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2021.12.014

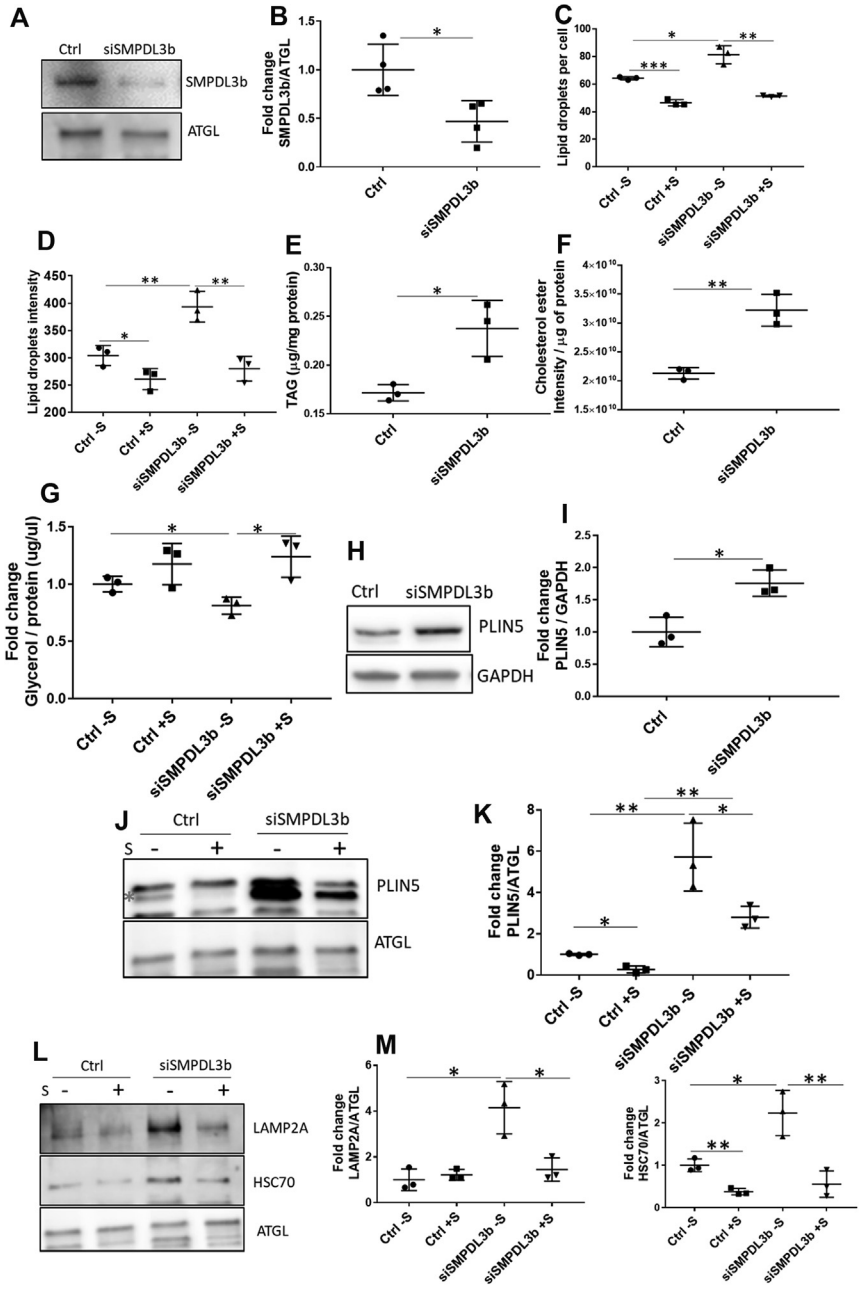


**Figure 1** PLIN5 mediates the accumulation of lipid droplets in SMPDL3b silenced human podocytes. **(A)** Western blot analysis of the protein expression of SMPDL3b in lipid droplets isolated from control (Ctrl) and SMPDL3b silenced (siSMPDL3b) human podocytes. **(B)** Bar graph representation of the fold change in protein expression of SMPDL3b. **(C)** Number and **(D)** intensity of lipid droplets is increased in siSMPDL3b human podocytes when compared to Ctrl human podocytes. Lipid droplet number and intensity are decreased in both ctrl and siSMPDL3b human podocytes treated with 10 μM Selonsertib when compared with untreated podocytes, respectively. **(E)** Triglycerides (TAG) and **(F)** cholesterol ester (CE) levels, determined by liquid chromatography mass spectrometry analysis (LC-MS), are increased in siSMPDL3b podocytes. **(G)** Lipolysis is decreased in siSMPDL3b when compared to Ctrl podocytes and increased in 10 μM Selonsertib treated siSMPDL3b when compared to untreated siSMPDL3b podocytes. **(H)** Western blot and **(I)** Bar graph representation of the fold change in protein expression of PLIN5 in lysates of human podocytes. **(J)** Western blot showing the expression of PLIN5 (shown with red asterisks) in lipid droplets of Ctrl and siSMPDL3b human podocytes. **(K)** Bar graph representation of the fold change in protein expression of PLIN5 normalized to ATGL. PLIN5 expression is increased in siSMPDL3b compared to Ctrl podocytes. Selonsertib (10 μM) treatment reduces the expression of PLIN5 in both Ctrl and siSMPDL3b podocytes when compared to untreated Ctrl and siSMPDL3b podocytes. **(L)** Western blot and **(M)** bar graph representation of the fold change in protein expression of LAMP2A and HSC70 normalized to ATGL. LAMP2A and HSC70 expression is increased in siSMPDL3b compared to Ctrl human podocytes. Selonsertib (10 μM) treatment reduced the expression of both LAMP2A and HSC70 in siSMPDL3b when compared with untreated siSMPDL3b podocytes. S = Selonsertib, $n$ = at least 3, $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$ by two-tailed $t$-test.

uptake and lipolysis were determined siSMPDL3b human podocytes and Ctrl podocytes. We found increased FA uptake (Fig. S3) and decreased lipolysis (Fig. 1G) in siSMPDL3b human podocytes when compared to Ctrl podocytes. To determine which FA transporter may contribute to increased FA uptake, mRNA expression analysis was performed. We found increased expression of the FA transporters FATP3, FATP5, FABP5 and FABP7 (Fig. S4) in siSMPDL3b podocytes while the expression of CD36 remained unchanged (Fig. S5). These data indicate that SMPDL3b deficiency in human podocytes affects FA uptake and lipolysis, leading to LD accumulation.

PLIN5 is a LD associated protein that is highly expressed in metabolically active and fatty acid oxidizing tissues like brown fat, heart, liver and skeletal muscle. PLIN5 promotes FA uptake and inhibits lipolysis, hence, removal of PLIN5 from LDs is essential for normal lipolysis. To investigate a possible role of PLIN5 in the inhibition of lipolysis in siSMPDL3b podocytes, PLIN5 expression levels were determined. We found increased PLIN5 mRNA (Fig. S6) and protein expression (Fig. 1H, I) in siSMPDL3b when compared to Ctrl podocytes as well as in LDs isolated from siSMPDL3b podocytes (Fig. 1J, K). These results suggest that decreased lipolysis in siSMPDL3b human podocytes could be mediated by increased expression of PLIN5.

To further investigate the role of PLIN5 in SMPDL3b deficiency-mediated LD accumulation and the inhibition of lipolysis, human podocytes were treated with Selonsertib, an inhibitor of PLIN5.[4] As expected, we found that Selonsertib treatment reduced PLIN5 expression (Fig. 1J, K) and decreased the number and intensity of LDs in both control and siSMPDL3b human podocytes (Fig. 1C, D). Lipolysis was restored to normal levels in siSMPDL3b podocytes treated with Selonsertib (Fig. 1G). These data indicate that SMPDL3b deficiency increases PLIN5 expression, which, in turn, leads to LD accumulation and decreased lipolysis.

Chaperon mediated autophagy (CMA) plays an important role in the removal of LD associated proteins. PLIN5 degradation at the LD surface through CMA was shown to be essential for lipolysis[5] and dysfunction of CMA is associated with several human diseases, including kidney diseases. Hence, we hypothesized that the expression of proteins involved in CMA will be decreased in LDs of siSMPDL3b human podocytes. Surprisingly, we found increased expression of heat shock cognate protein (HSC70) and of lysosome-associated membrane protein 2A (LAMP2A) in LDs of siSMPDL3b human podocytes (Fig. 1L, M). The increase in the expression of these CMA proteins could indicate that 1) the interaction of SMPDL3b with PLIN5 is necessary to prevent the interaction of PLIN5 with other proteins at the LDs surface, or 2) that SMPDL3b may function as one of the tethers required for the proper binding of LDs to lysosomes to allow for efficient degradation of LDs surface proteins by CMA.

Finally, we determined if inhibition of PLIN5 expression with Selonsertib is sufficient to prevent the increased expression of HSC70 and LAMP2A in LDs. As expected, Selonsertib treatment reduced the expression of HSC70 and LAMP2A in LDs of siSMPDL3b podocytes compared to untreated as well as HSC70 expression in control podocytes (Fig. 1L, M). These data indicate that increased presence of HSC70 and LAMP2A in LDs of siSMPDL3b podocytes is due to increased PLIN5 expression. The observation that the presence of HSC70 and LAMP2A is decreased in LDs treated with Selonsertib suggest that PLIN5 regulates HSC70 and LAMP2A expression. Similarly, increased PLIN5 expression in siSMPDL3b podocytes causes increased HSC70 and LAMP2A protein expression, which is likely a consequence of inefficient degradation of PLIN5 by CMA.

## Conflict of interests

A.F. and S.M. are inventors on pending (PCT/US2019/032215; US 17/057,247; PCT/US2019/041730; PCT/US2013/036484; US 17/259,883; US17/259,883; JP501309/2021, EU19834217.2; CN-201980060078.3; CA2,930,119; CA3,012,773,CA2,852,904) or issued patents (US10,183,038 and US10,052,345) aimed at preventing and treating renal disease. They stand to gain royalties from their future commercialization. A.F. is Vice-President of L&F Health LLC and is a consultant for ZyVersa Therapeutics, Inc. ZyVersa Therapeutics, Inc has licensed worldwide rights to develop and commercialize hydroxypropyl-beta-cyclodextrin from L&F Research for the treatment of kidney disease. A.F. also holds equities in Renal 3 River Corporation. SM holds indirect equity interest in, and potential royalty from, ZyVersa Therapeutics, Inc. by virtue of assignment and licensure of a patent estate. A.F. and S.M. are supported by Aurinia Pharmaceuticals Inc. and by Boehringer Ingelheim.

## Funding

This project was supported by grants from National Institutes of Health (No. R01DK117599, R01DK104753 and R01CA227493) to A.F. and S.M, and the Miami Clinical Translational Science Institute (No. U54DK083912, UM1DK100846, U01DK116101 and UL1TR000460) to A.F, and the Ophthalmology mass spectrometry facility, Vision Core grant (No. EY14801).

## Appendix A.Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2021.12.014.

## References

1. Mitrofanova A, Molina J, Varona Santos J, et al. Hydroxypropyl-β-cyclodextrin protects from kidney disease in experimental Alport syndrome and focal segmental glomerulosclerosis. *Kidney Int*. 2018;94(6):1151–1159.
2. Mallela SK, Mitrofanova A, Merscher S, Fornoni A. Regulation of the amount of ceramide-1-phosphate synthesized in differentiated human podocytes. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2019;1864(12):158517.
3. Mitrofanova A, Mallela SK, Ducasa GM, et al. SMPDL3b modulates insulin receptor signaling in diabetic kidney disease. *Nat Commun*. 2019;10(1):2692.

4. Tan Y, Jin Y, Wang Q, Huang J, Wu X, Ren Z. Perilipin 5 protects against cellular oxidative stress by enhancing mitochondrial function in HepG2 cells. *Cells*. 2019;8(10):1241.
5. Ma SY, Sun KS, Zhang M, et al. Disruption of Plin5 degradation by CMA causes lipid homeostasis imbalance in NAFLD. *Liver Int*. 2020;40(10):2427—2438.

Shamroop Kumar Mallela[a,b], Mengyuan Ge[a,b], Judith Molina[a,b], Javier Varona Santos[a,b], Jin-Ju Kim[a,b], Alla Mitrofanova[a,b], Hassan Al-Ali[a,b], Brian Marples[c], Sandra Merscher[a,b], Alessia Fornoni[a,b,*]

[a] *Katz Family Division of Nephrology and Hypertension, Department of Medicine, University of Miami, Miller School of Medicine, Miami, FL 33136, USA*

[b] *Peggy and Harold Katz Family Drug Discovery Center, University of Miami, Miller School of Medicine, Miami, FL 33136, USA*

[c] *Department of Radiation Oncology, University of Rochester, Rochester, NY 14627, USA*

*Corresponding author. Katz Family Division of Nephrology and Hypertension, Peggy and Harold Katz family Drug Discovery Center, University of Miami, 1580 NW 10th Ave, Miami, FL 33136, USA. Fax: +1 305 243 3506.
*E-mail address:* afornoni@med.miami.edu (A. Fornoni)

24 August 2021
Available online 3 February 2022