The Role of Oxysterol-Binding Protein Like 7 in Podocyte Health: Implications in Chronic Kidney Disease Pathophysiology

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WCN24-AB-1285

Background

ER Stress and Podocytes

The endoplasmic reticulum (ER) is a crucial cellular organelle, central to protein folding, lipid biosynthesis, and calcium storage. Under conditions of physiological stress, proteins may misfold, leading to an accumulation of unfolded proteins within the ER. This accumulation activates an adaptive cellular response known as the unfolded protein response (UPR) aimed at restoring ER homeostasis. However, dysregulation of UPR signaling pathways, including IRE1α, PERK, and ATF6, has been associated with injury to podocytes, critical cells in renal filtration. Such dysregulation is believed to play a significant role in the advancement of kidney diseases [1,2].

Lipid Dysregulation in Podocytes

Emerging research highlights the impact of lipid metabolism on the health of podocytes. Disruptions in lipid homeostasis can result in the accumulation of lipid droplets within cells, a condition termed "lipotoxicity." This exacerbates ER stress, further promoting cellular apoptosis and contributing to the pathology of renal diseases [3,4].

OSBPs and OSBPL7

Oxysterol-binding proteins (OSBPs), and their related family members such as OSBPL7, are pivotal in maintaining lipid transport and cellular homeostasis. These proteins mediate the exchange of phosphatidylinositol-4-phosphate (PI4P) and cholesterol between various cellular membranes, including the ER and the Golgi apparatus [5,6]. The activity of OSBPs is critically dependent on the concentration of PI4P [7,8], playing a key role in establishing a PI4P gradient that facilitates the exchange of cholesterol/PI4P, thus supporting lipid transport processes [9,10]. Disturbances in this gradient can impair critical cellular functions, including autophagy and protein folding at the ER membrane [11-13]. In the context of podocytes, OSBPL7 specifically influences the expression and function of the ATP Binding Cassette Subfamily A Member 1 (ABCA1), which is essential for cholesterol efflux [4]. Therefore, OSBPs, including OSBPL7, are integral to ER protein quality control, suggesting their potential role in conditions leading to ER stress and associated pathologies such as podocytopathies, chronic kidney disease (CKD), liver disease, diabetic kidney disease (DKD), and acute kidney injury (AKI) [3,4].

Aims

The objective of this study is to elucidate the role of OSBPL7 deficiency in the progression of CKD. By employing murine and zebrafish models, along with cultured podocytes, we aim to explore how the deficiency of OSBPL7 contributes to ER stress, lipid dysregulation, and ultimately, podocyte apoptosis.

Methods

Experimental Models and Cell Culture

Two murine models (Col4a3-/- and db/db mice) and L-fabp:DBP-eGFP zebrafish were used for in vivo studies, all maintained under pathogen-free conditions with approved protocols. Conditionally immortalized mouse podocytes, including stable OSBPL7 deficient (SiOSBPL7) podocytes generated via siRNA, were cultured for in vitro studies.

Assays and Analyses

Biochemical assays such as Western blotting were used to analyze specific proteins. Antibodies probed include OSBPL7, PERK, IRE1a, BiP, phosphorylated SAPK/JNK (p-SAPK/JNK), and total SAPK/JNK. Apoptosis was quantified using the Caspase-Glo® 3/7 Assay. Membrane fluidity, as well as lipid and fatty acid concentrations, were assessed using specialized kits from Abcam and Invitrogen. Full-length OSBPL7 was introduced into podocytes using Fugene 6 for lentiviral transfection.

Ultrastructural Analysis

Transmission Electron Microscopy (TEM) was employed to examine the ultrastructure of zebrafish glomeruli.

Results

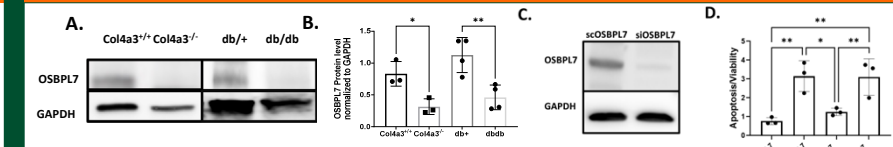

Figure 1: OSBPL7 protein levels are reduced in Col4a3-/- and db/db mouse kidney cortex. A) Representative western blot images of OSBPL7 levels with B) quantification. C) Stable OSBPL7 deficient (SiOSBPL7) podocytes were generated using siRNA. D) Apoptosis levels increase in OSBPL7 deficient podocytes and return to control levels with transfection of full-length OSBPL7 (OSBPL7FL) but not with transfection of OSBPL7 plasmid containing a deletion of the FFAT domain (OSBPL7FFAT-). [N=3; *P<0.05, **P<0.01]

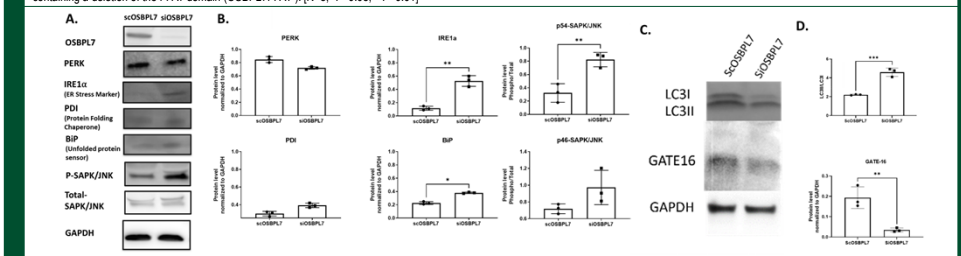

Figure 2: OSBPL7 deficiency leads to ER stress and decreased autophagic flux A) Representative western blot images of PERK, IRE1a, PDI, phospho and total-SAPK/JNK, BiP, and GAPDH with B) quantification from 3 independent experiments. C) Representative western blot images of LC3I, LC3II, and GATE16 levels with GAPDH loading control with D) quantification from 3 independent experiments. [N=3; *P<0.05, **P<0.01, ***P<0.005]

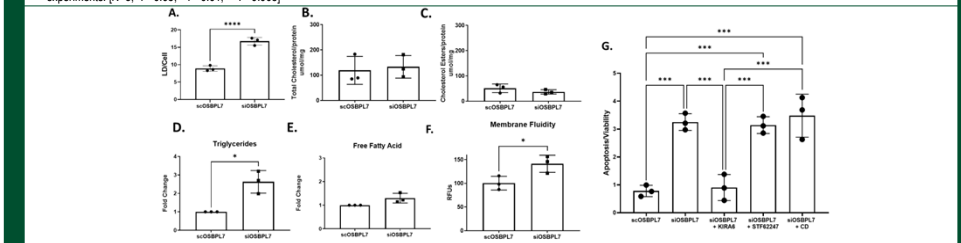

Figure 3: Increased ER Stress and not Decreased Autophagy or increased lipid droplets is Responsible for Apoptosis in OSBPL7 Deficient Podocytes. A) LD quantification per cell in siOSBPL7 podocytes vs control. B) Total cholesterol and C) cholesterol ester levels were not changed between siOSBPL7 and scOSBPL7 podocytes. D) Triglycerides are increased in siOSBPL7 podocytes while E) Free fatty acids are not changed as indicated by fold change from siOSBPL7 compared to scOSBPL7 levels. F) Membrane fluidity is increased in siOSBPL7 podocytes compared to scOSBPL7. (N=3; *P<0.05, ****P<0.001) G) Apoptosis levels increase in OSBPL7 deficient podocytes and return to control levels when treated with KIRA6, an inhibitor of IRE1a, but treatment with STF62247, an autophagy inducer, or Cyclodextrin (CD), an inhibitor of lipid droplets, does not, indicating the effect on apoptosis is driven through ER stress by IRE1a activation. (N=3; ***P<0.005)

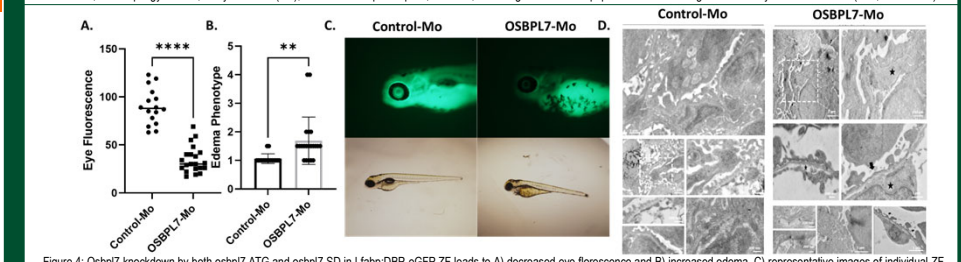

Figure 4: Osbpl7 knockdown by both osbpl7 ATG and osbpl7 SD in l-fabp:DBP-eGFP ZF leads to A) decreased eye florescence and B) increased edema. C) representative images of individual ZF. D) Transmission electron microscopy (TEM) images of zebrafish glomerulus at 5 DPI with either Control-Mo and osbpl7-Mo. Substantial glomerular damage is conspicuous in the osbpl7 -Mo injected larvae. The detailed TEM analysis reveals the loss of endothelial fenestrations (white arrows) and widening and rupture of the glomerular basement membrane (GBM) (star). The distinct alterations in podocyte structures are shown, including podocyte effacement and protrusions of foot processes into the GBM (Black arrows), a common hallmark of proteinuric states in fish. These observations point to clear podocyte and glomerular damage, in alignment with the proteinuria documented in the fish. [4 DPI; n=16; **P<0.01, ****P<0.001]

Key Findings

Our investigation into OSBPL7's impact on CKD, utilizing murine and zebrafish models alongside cultured podocytes, yielded noteworthy insights:

OSBPL7 and CKD: There was a noted correlation between reduced OSBPL7 expression and increased podocyte injury in mouse CKD models (Col4a3-/- and db/db).

ER Stress Markers: OSBPL7 deficiency resulted in elevated ER stress markers and the activation of the SAPK/JNK pathway, indicating a targeted activation of the IRE1α-mediated UPR pathway.

Mechanism of Injury: The study identified ER stress as the principal mechanism behind podocyte apoptosis due to OSBPL7 deficiency, with lipid dysregulation and autophagy alterations observed but not directly linked to apoptosis. The intervention with IRE1α inhibitor KIRA6 reversed apoptosis, highlighting a therapeutic avenue.

Zebrafish Model Validation: Knockdown of OSBPL7 in zebrafish models recapitulated human CKD phenotypes, including proteinuria and glomerular damage, further affirming OSBPL7's essential role in renal health.

Conclusions

The Pivotal Role of OSBPL7

This study reveals the crucial role of OSBPL7 in modulating ER stress and maintaining podocyte health, making it a potential target for therapeutic interventions in CKD and related renal disorders.

Therapeutic Potential of OSBPL7

The direct association between OSBPL7 deficiency and increased ER stress leading to podocyte apoptosis underlines the therapeutic potential of targeting OSBPL7 pathways or their downstream effects.

Conservation Across Species

The conservation of OSBPL7's role across species from zebrafish to mammals emphasizes its fundamental importance in kidney physiology and pathology, suggesting further research into OSBPL7 modulation or ER stress mitigation as promising approaches for treating CKD and potentially other related conditions.

Acknowledgements

This research was made possible through the support of multiple grants and organizations:

- A.F. and S.M. are supported by NIH grant R01DK136679.
- Additional NIH funding for A.F. included grants U54DK083912, UM1DK100846, U01DK116101, and UL1TR000460 from the Miami Clinical Translational Science Institute.
- J.P. is supported by NIH F32 1F32DK135187.
- J.P. was also previously funded by the American Society of Nephrology Ben J. Lipps Research Fellowship Program for a portion of this work.

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