RTN3 interacted with FABP5 and promoted lipid droplet biogenesis in cardiomyocytes

D. Guo1, M.M. Zhang2, B.C. Qi2, T.W. Peng2, M.C. Liu2, FENG Fu3, YING Wang2, LANG Hu2, YAN Li2

1Fourth Military Medical University, Xi'an, China
2Fourth Military Medical University, Department of Cardiology, Tangdu Hospital, Xi'an, China
3Fourth Military Medical University, Department of Physiology and Pathophysiology, Xi'an, China

Funding Acknowledgements: Type of funding sources: Public grant(s) – National budget only. Main funding source(s): National Natural Science Foundation of China

Background: Lipid droplet (LD) accumulation is a notable feature of obesity-induced cardiomyopathy; however, detailed processes governing cardiac LD content remains poorly understood. Reticulon 3 (RTN3), a tubular endoplasmic reticulum (ER) localized protein, was recently identified as a key modulator of lipid metabolism in adipocytes. But its role in cardiac lipid metabolism and the specific mechanism of RTN3 regulating lipid content are currently unknown.

Purpose: To investigate the role of RTN3 in cardiac LD accumulation and the mechanism by which RTN3 affects LD metabolism.

Methods: We labelled LD, free fatty acids (FFA), and nucleus in cardiomyocytes with fluorescent markers and upregulated/downregulated RTN3 expression with adenovirus. The effect of RTN3 on cardiac LD content and biogenesis was detected with confocal microscopy. Then we investigated the interaction partner of RTN3 with co-immunoprecipitation (Co-IP), liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, and immunofluorescence staining. The function of the complex which RTN3 formed with its interaction protein was then explored.

Results: RTN3 overexpression significantly increased LD content in cardiomyocytes, which phenocopied the effects of palmitate stress. In contrast, palmitate-induced LD accumulation was blocked by RTN3 knockdown. Tubular ER, where RTN3 resides, is the primary organelle regulating LD biogenesis. Based on that, we hypothesized that the effect of RTN3 on LD content could be attributed to its direct effect on LD biogenesis in the ER. Further FFA tracing results demonstrated that RTN3 overexpression and palmitate could markedly increase FFA-LD colocalization, suggesting the LD biogenesis was activated, but this increase in colocalization was absent in cardiomyocytes infected with Adsh-RTN3. Live cell imaging also revealed that upregulation of RTN3 significantly accelerated cardiac LD biogenesis and downregulation of RTN3 suppressed palmitate-induce LD biogenesis. Then we investigated the interaction partner of RTN3 and found that RTN3 formed a complex with fatty acids binding protein 5 (FABP5), which is an intracellular carrier for long-chain FFAs. Mechanistically, RTN3 directly bound with FABP5, which facilitated the directed transport of FFA to ER and thus promoted LD biogenesis. FABP5 knockdown blocked RTN3-mediated LD biogenesis. Moreover, we discovered that the 1st-194th amino acids of RTN3 was responsible for interacting with FABP5 and promoting LD biogenesis.

Conclusions: Our study reveals a novel mechanism contributing to LD biogenesis in cardiomyocytes. Based on our results, manipulating LD biogenesis by modulating RTN3 may be a potential strategy for treating LD accumulation in obese patients.
RTN3 formed a protein complex with FABP5