Title	Adipocyte fatty acid binding protein potentiates toxic lipids-induced endoplasmic reticulum stress via its inhibition of autophagy
Author(s)	Hoo, RLC; Lee, PC; Lam, KSL; Xu, A
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Adipocyte fatty acid binding protein potentiates toxic lipids-induced endoplasmic reticulum stress via its inhibition of autophagy

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RLC Hoo, IPC Lee, KSL Lam, A Xu
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Chronic inflammation is the key link between obesity and its related cardio-metabolic complications. Endoplasmic reticulum (ER) stress is the potent trigger of inflammation in obese adipose tissue. However, the mechanism that links ER stress with inflammation is unclear. Adipocyte fatty acid binding protein (A-FABP) has been shown to mediate endotoxin-induced inflammation in macrophages by forming a positive loop with c-Jun-N terminal kinase (JNK) which is the downstream regulator of ER stress. This study aimed to examine the role of A-FABP in association with autophagy in potentiating toxic lipids-induced ER stress in macrophages.

Methods: RAW264.7 macrophages with adenovirus-mediated over-expression of A-FABP or luciferase, and primary macrophages derived from A-FABP knockout mice or their WT littermates were treated with palmitic acid (PA) or vehicle. RAW264.7 macrophages were transfected with siRNA of autophagic protein Atg7 or scramble RNA followed by the stimulation of PA. The autophagic flux, mRNA and protein expression of ER stress markers, autophagic proteins and inflammatory markers were determined by real-time quantitative PCR and Western blot analysis.

Results: Adenovirus-mediated over-expression of A-FABP reduced the expression of autophagic proteins Atg7 and beclin-1, and this change was accompanied by enhanced ER stress and inflammation in response to PA stimulation. Both basal and PA-induced autophagic flux and protein expression were enhanced while ER stress was reduced in macrophages derived from A-FABP knockout mice comparing to wild type macrophages. Knocking down of Atg7 leading to defective autophagy elevated PA-induced ER stress in macrophages. Treatment of macrophages with JAK2 inhibitor AG490 also reduced the autophagic protein expression, but further enhanced the ER stress in response to PA stimulation.

Conclusion: A-FABP potentiates toxic lipids-induced ER stress through inhibition of autophagy which may possibly via its attenuation of JAK2 signalling pathway in macrophages.

Effects of functional transient receptor potential channels on adipogenesis and proliferation in human preadipocytes

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C Hui, GR Li
Department of Medicine, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong

Background: Preadipocytes are extensively used as a type of proliferative cell culture model to investigate proliferation and differentiation of adipocytes and lipodystrophy (eg obesity)-related metabolic dysfunctions and disorders. However, cell biology is not well understood in human preadipocytes. The present study was to investigate the expression of transient receptor potential (TRP) channels in human preadipocytes, and their role in regulating adipogenesis and proliferation.

Methods: Whole-cell patch voltage-clamp, RT-PCR, Western blot, and confocal microscopic approaches were used to determine functional expression of TRP channels in cultured human preadipocytes. ShRNA targeting TRP channels were constructed to silence the related TRP channels. Adipogenesis and oil red O staining were applied to observe the effect of the TRP channels on cell differentiation. Cell proliferation assay was made with MTT and ^{3}H-thymidine incorporation approaches.

Results: A small background current was inhibited by the TRPC channel blocker La^{3+}. Removal of Mg^{2+} of pipette solution or bath solution induced a Mg^{2+}-sensitive current, and the current was suppressed by the TRP channel blocker 2-aminoethoxydiphenyl borate. In addition, an intracellular Ca^{2+}-activated current was inhibited by the TRPV channel blocker capsazepine. RT-PCR revealed significant mRNA expression of TRPC1, TRPC4, TRPV2, TRPV4, and TRPM7 channels in human preadipocytes. Western blot analysis confirmed the protein expression of these TRP channels. Interestingly, shRNAs targeting TRPV2, TRPV4 and TRPM7 suppressed the corresponding gene and protein expression, and significantly reduced adipogenesis of human preadipocytes, which was revealed by the reduced oil red O staining and the decreased expression of peroxisome proliferator-activated receptor gamma (PPARγ, a marker of adipogenesis). Proliferation of human preadipocytes was reduced by TRPV2-shRNA, TRPV4-shRNA and TRPM7-shRNA.

Conclusion: Our results demonstrate for the first time that multiple TRP channels, TPC1/4, TRPV1/2/4, and TRPM7, are present in human preadipocytes. TRPV2, TRPV4 and TRPM7 channels participate in regulating adipogenesis and proliferation.