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Ezetimibe ameliorates renal fibrosis via Nrf2-related pathway

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Objectives: Nuclear factor 2-related factor 2 (Nrf2) has a protective role against various celluar injuries by inflammatory reaction and oxidative stress. The activity of Nrf2 is regulated by Kelch-like ECH-assocaited protein 1 (Keap1) which promotes ubiquitylation and degradation of Nrf2. SQSTM1/p62, an adaptor protein of autophagies is another regulator of Nrf2 which activates Nrf2 nuclear translocation by autophagic degradation of Keap1. It has been recently known that ezetimibe activates Nrf2 via p62-related pathway and has a protective effect against the progression of hepatic fibrosis. In this study, we evaluated whether the Nrf2 pathway was related to renal tubulointerstitial fibrosis in an animal model, and whether ezetimibe has protective effect against fibrosing tubular epithelial injury in cultured renal tubular cells.

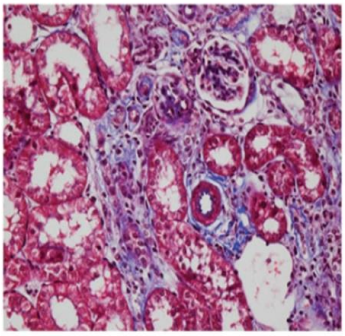
Methods: To induce renal tubulointerstitial fibrosis, C57 mice were injected with folic acid or vehicle intraperitoneally. Kidneys were harvested 4 weeks after folic acid injection. Silencing of p62 was performed by CRISPR/Cas9 system in cultured HK-2 cells. Both wild type and p62-silenced HK-2 cells were treated with transforming growth factor-β (TGF-β), ezetimibe, or both TGF-β and ezetimibe for 18 hours. Cells were harvested and the amount of collagen I was measured.

Results: Folic acid-treated mice showed significant tubulointerstitial fibrosis confirmed by histologic examination and increased smooth muscle actin expression. The expression of Keap1 and p62 also increased in folic acid-treated mice confirming that the Nrf2-Keap1 pathway was related to tubulointerstitial fibrosis. HK-2 cells treated with TGF-β showed markedly increased collagen I expression which was ameliorated by ezetimibe treatment. Silenicing of p62 resulted in more collagen 1 expression after TGF-β treatment and less effective function of ezetimibe.

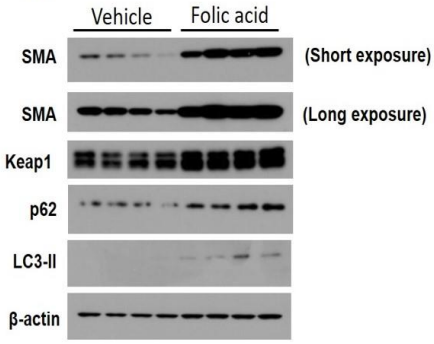
Conclusions: The progression of tubulointerstitial fibrosis is related to Nrf2-Keap1 pathway. The effect of TGF-β-induced tubular injury can be ameliorated by ezetimbe treatment and p62-related Nrf2-Keap1 pathway is responsible to this process.

Intraperitoneal folic acid injection induced renal fibrosis (A) which was associated with increased Keap1 and p62 expression (B). TGF-b challenge on cultured HK-2 cells resulted in increased production of collagen I which was reduced by adding ezetimibe. This effect was ameliorated by p62 silencing (C).

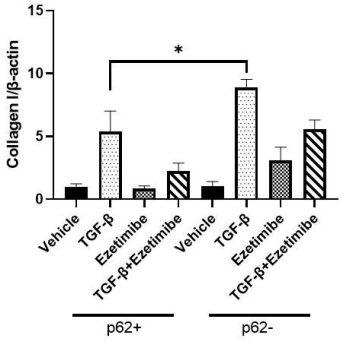
(A)



(B)


Vehicle
Folic acid
SMA
(Short exposure)
SMA
(Long exposure)
Keap1
p62
LC3-II
β-actin

(C)


Collagen I/β-actin
15
10
5
0
*
Vehicle
TGF-β
Ezetimibe
TGF-β+Ezetimibe
Vehicle
TGF-β
Ezetimibe
TGF-β+Ezetimibe
p62+
p62-