# Iron overload inhibits cell proliferation and promotes autophagy via PARP1/SIRT1 signaling in endometriosis and adenomyosis

Zhou Y, Zhao X, Zhang L, Xia Q, Peng Y, Zhang H, Yan D, Yang Z, Li J.Toxicology. 2021 Nov 23;465:153050. doi: 10.1016/j.tox.2021.153050. Online ahead of print.PMID: 34826546

## Abstract

Emerging evidence suggests that excess iron accumulates in endometriotic and adenomyotic lesions. However, the role iron overload plays in the pathogenesis of endometriosis or adenomyosis remains unknown. Primary human eutopic endometrial stromal cells (EuESCs) from endometriosis or adenomyosis patients were used as the in vitro model of endometriosis or adenomyosis in this study. We found that iron, manifesting as ferric ammonium citrate (FAC; 0.05-4.8 mM), significantly inhibited cell growth, induced oxidative stress through the Fenton reaction, and functionally activated autophagy in EuESCs, as measured by 5-ethynyl-2'-deoxyuridine incorporation assay, MitoSOX™ Red staining, LC3 turnover assay, and tandem mCherry-eGFP-LC3B ﬂuorescence microscopy. Immunohistochemistry analysis of Ki67 expression in proliferative-phase endometrial tissues revealed that cell proliferation in ectopic tissues was dramatically compromised, suggesting that iron overload may play a role in cell growth inhibition in vivo. We observed that autophagy may alleviate the FAC-induced inhibition of endometrial stromal cell proliferation. Furthermore, sequential FAC (0.8 mM, 24 h) and hydrogen peroxide (H2O2; 300 μM, 2 h) treatment successfully induced the Fenton reaction in EuESCs and caused extensive apoptosis, whereas the disruption of autophagy by the knockdown of BECN1 further aggravated cell death. MitoSOX™ Red staining showed that autophagy may promote the survival of EuESCs by decreasing of the Fenton reaction-induced reactive oxygen species generation. In addition, we observed that the Fenton reaction-induced oxidative stress significantly suppressed iron overload-induced autophagy. Moreover, we found that FAC treatment impaired poly(ADP-ribose)-polymerase 1 (PARP1) expression while simultaneously upregulating SIRT1 expression in EuESCs. Our data further showed that PARP1 expression decreased in endometriotic lesions, which may partially result from iron overload. We also found that PARP1 inhibition aggravated iron overload-induced cell growth suppression, and was implicated in iron overload-induced autophagy. In addition, SIRT1 silencing alleviated iron overload-induced PARP1 downregulation and autophagy activation. Overall, our data suggest that iron overload in endometrial stromal cells of endometriotic or adenomyotic lesions may be involved in the inhibition of cell proliferation, simultaneously with the activation of protective autophagy via PARP1/SIRT1 signaling.

**Keywords:**Adenomyosis; Autophagy; Endometriosis; Fenton reaction; Iron overload; PARP1/SIRT1.