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Loss of Extended Synaptotagmin 2 expression enhances cigarette smoke-induced plasma membrane damage in lung epithelial cells

Maintaining plasma membrane (PM) integrity is essential for cellular homeostasis. Cigarette smoke and its constituents compromise the PM integrity of lung cells, necessitating rapid damage evasion and repair processes. Ca^{2+} influx serves as a key trigger for PM repair through lysosomal exocytosis, microparticle shedding, patch formation, or endocytosis. We have utilized a yeast deletion screen to identify Ca^{2+}-binding tricalbins, which are mammalian homologs of extended synaptotagmins (ESyts), as key PM repair genes. Subsequently, the role of ESyt1–3 proteins in lung epithelial cell PM repair were investigated. To achieve this, lentiviral short hairpin RNA (shRNA) approaches were utilized to knock down the expression of ESYT1–3 genes in A549 cells. Scrambled and ESYT1–3 shRNA-transfected A549 cells were exposed to either cigarette smoke extract (CSE) or saponin, a nonionic detergent, under both Ca^{2+}-containing and Ca^{2+}-depleted conditions. PM damage was assessed using a SYTOX Orange permeability assay. Exposure to 2.5% CSE induced PM damage approximately 2.2-, 2.3-, and 1.9-fold higher in A549 cells transfected with ESYT1, ESYT2, and ESYT3 shRNA, respectively, compared to scrambled shRNA-transfected A549 cells. Similarly, silencing each ESYT gene enhanced saponin-induced PM damage. Cells transfected with ESYT2 shRNA showed the highest PM damage with both CSE and saponin. CSE (5%) exposure increased the expression of the ESYT genes in a Ca^{2+}-dependent manner in A549 cells. Collectively, these findings underscore the critical role of ESYT genes, particularly ESYT2, in maintaining PM integrity and highlight the need for further investigation into PM repair mechanisms in pulmonary diseases.