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4-Phenyl Butyric Acid (PBA) Promotes Aggregate Formation in HEK 293 Cells Expressing Wild Type or Mutant Pulmonary Surfactant Protein C (SP-C)

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Pulmonary surfactant protein C (SP-C) is a hydrophobic lipid associated protein secreted by alveolar type 2 cells that reduces alveolar surface tension during gas exchange. Accumulation of mutant SP-C proproteins, SP-$C^{\Delta exon4}$ or SP-C^{L188Q}, in endoplasmic reticulum (ER) leads to ER stress that may contribute to the pathogenesis of interstitial lung disease. 4-phenyl butyric acid (PBA) is a chemical chaperone that can reduce retention of mutant and misfolded proteins in ER, thereby decreasing protein aggregation and accumulation-associated cell stress. In this study, we tested whether PBA reduces accumulation of wild type (WT) and mutant SP-C protein aggregates in HEK 293 cells stably expressed SP-C^{WT197}, SP-C^{L188Q}, and SP-$C^{\Delta exon4}$ with or without PBA incubation.

Electron dense aggregates detected by electron microscopy localized to ER, perinuclear inclusions, and lysosomes of control HEK 293 cells expressing WT and mutant SP-C (Figure 1A-1C). At least 50% of aggregates detected in control HEK 293 cells were less than 0.2 µm in diameter. In the presence of 1 mM PBA, pronounced aggregate formation was detected for all three HEK 293 cell lines expressing WT and mutant SP-C (Figure 1D-1F). Aggregate distribution analyzed by categorized rank test determined that there was a significant increase in the number of aggregates greater than 0.2 µm in PBA-treated HEK 293 cells when compared to control cells ($p<0.001$). The increase in aggregate formation was primarily associated with an increase in the number of aggregates between 0.2 to 0.4 µm. Although aggregate formation was also increased for aggregates greater than 0.4 µm, they were not statistically significant.

In addition to pronounced aggregate formation, PBA-treated mutant SP-C expressing HEK 293 cells often had dilated ER cisternae and mitochondria compared to control cells. Localization of SP-C proprotein by immuno EM detected focal localization of SP-C proprotein to the ER lumen, perinuclear electron dense inclusions, multivesicular bodies, and lysosomes of PBA treated cells (Figure 2A-2C). These findings are consistent with Western blotting analyses in which PBA not only increased accumulation of mutant SP-C^{L188Q} and SP-$C^{\Delta exon4}$ proprotein in mutant SP-C expressing HEK 293 cells but also increased accumulation of wild type SP-C proprotein in HEK 293 cells expressing SP-C^{WT197}, suggesting that PBA facilitates stabilization of WT and mutant SP-C proproteins.

These results suggest that PBA does not relieve aggregations in HEK 293 cells expressing WT and mutant SP-C. In contrast, an increase in stabilization of WT and mutant SP-C proproteins induced by PBA leads to elevated levels of SP-C proproteins and cytotoxicity in transfected HEK 293 cells. How PBA interacts with ER and other compartments to stabilize WT and mutant SP-C proproteins will require further investigation.

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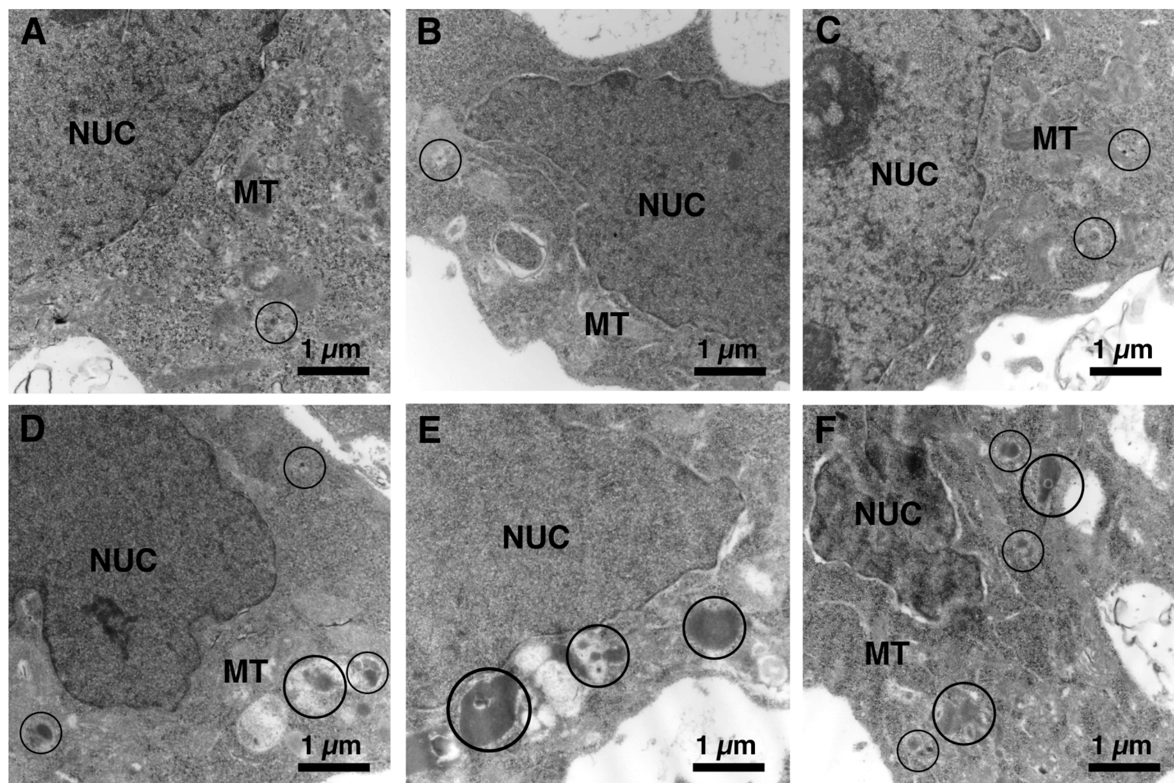

Figure 1. Localization of aggregates to HEK 293 cells expressing SP-C^{WT197}, SP-C^{L188Q} and SP-C$^{\Delta exon4}$ expressing HEK 293 cells with or without PBA. A. SP-C^{WT197}, buffer control. B. SP-C^{L188Q}, buffer control. C. SP-C$^{\Delta exon4}$, buffer control. D. SP-C^{WT197}, 1 mM PBA. E. SP-C^{L188Q}, 1 mM PBA. F. SP-C$^{\Delta exon4}$, 1 mM PBA. Note that there was a significant increase in aggregate formation (circle) in PBA treated HEK 293 cells. NUC: nucleus; MT: mitochondria.

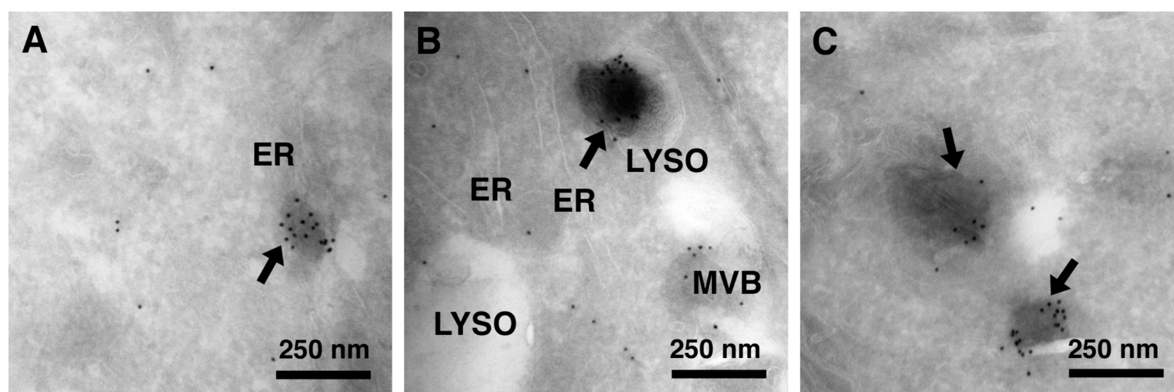

Figure 2. Localization of SP-C proprotein to 1 mM PBA-treated HEK 293 cells expressing SP-C^{WT197}, SP-C^{L188Q}, and SP-C$^{\Delta exon4}$. A. HEK 293 cells expressing SP-C^{WT197}. B. HEK 293 cells expressing SP-C^{L188Q}. C. HEK 293 cells expressing SP-C$^{\Delta exon4}$. Localization of SP-C proprotein was demonstrated by cryoimmunogold labeling using rabbit antisera directed against the N-terminus of SP-C proprotein and 10 nm protein A gold. Note that SP-C proprotein positive aggregates (arrow) localized to ER cisternae and perinuclear electron dense inclusions. ER: endoplasmic reticulm; LYSO: lysosome; MVB: multivesicular body.

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