Acute Toxicity of 1,3-Butadiene Exposure at the Air-Liquid Interface of Human Bronchial Epithelial Cells

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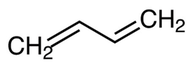
Abstract #4742

INTRODUCTION

1,3-Butadiene



- 1,3-Butadiene (1,3-BD) is a petroleum-derived volatile organic compound
- 36th highest volume chemical produced in the United States
- Primarily used for production of synthetic rubber (tires, resins, and plastics)
- Emitted from combustion sources:

  - OSHA limits: 1ppm over 8 hours, 5ppm over 15-minute period

In Humans:
- Hematopoietic cancers in workers[1,2]
- Cardiovascular disease risk[3]
  - vascular dysfunction[4]
  - biomarkers of cardiopulmonary injury & oxidative stress[5]

Animal Studies:
- Multi-organ tumorigenesis[3,6,7]
  - e.g., lung, liver, hematopoietic, mammary, & reproductive
- Exacerbation of atherosclerosis[8]

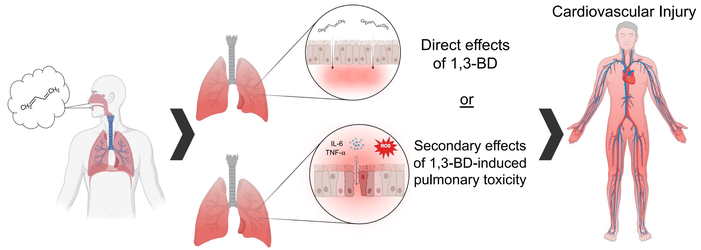


Figure 1: Potential mechanisms of 1,3-BD-induced Cardiovascular Injury

Hypothesis: 1,3-BD exposure increases epithelial barrier permeability & upregulates genes involved in pulmonary inflammatory and oxidative stress pathways, which may extend systemically to cardiovascular injury.

METHODS

Air-liquid interface (ALI) may provide a more physiologically relevant approach for modelling human inhalation exposures *in vitro*.

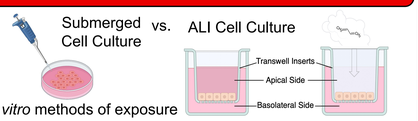
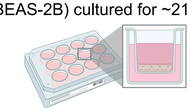
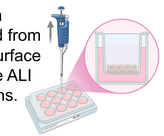


Figure 2: *In vitro* methods of exposure

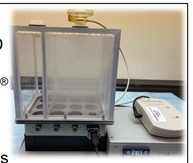
1. Human bronchial epithelial cells (BEAS-2B) cultured for ~21 days.



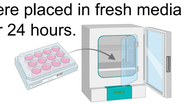
2. Media removed from apical surface to create ALI conditions.



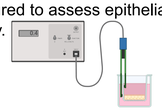
3. Generation & delivery of 1,3-BD (2%v/v) aerosol cloud via Vitrocell® cloud system.



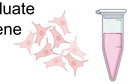
4. Following exposure, cells were placed in fresh media for 24 hours.



5. Transepithelial electrical resistance (TEER) measured to assess epithelial barrier integrity.



6. Media & cells collected to evaluate cytotoxicity & gene expression.



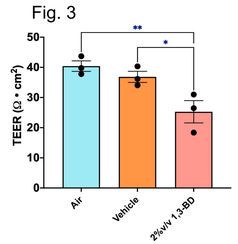
7. Messenger RNA sequencing (mRNA-seq) performed via Novogene Inc. to identify differentially expressed genes (DEGs) & pathways.

*n=3 independent experiments, each with n=6 technical replicates per group.

RESULTS

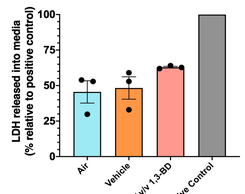
Figures 3 & 4: 1,3-butadiene reduced TEER of the cell monolayer (1.5-fold) without causing overt cell death.

Fig. 3



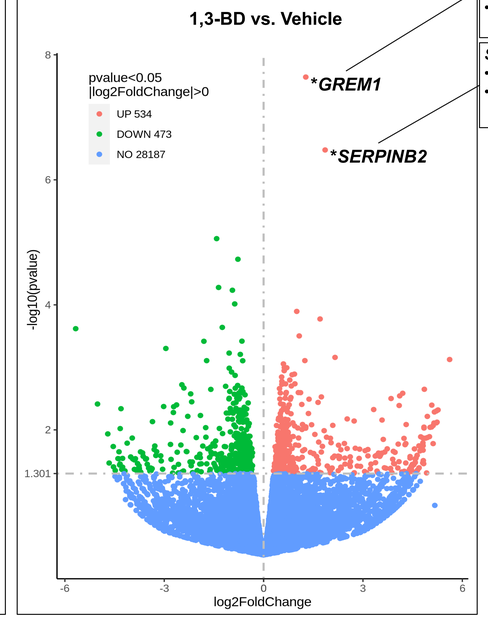
*Mean ± SEM, *P<0.05, **P<0.01.

Fig. 4



*%LDH normalized to positive control.

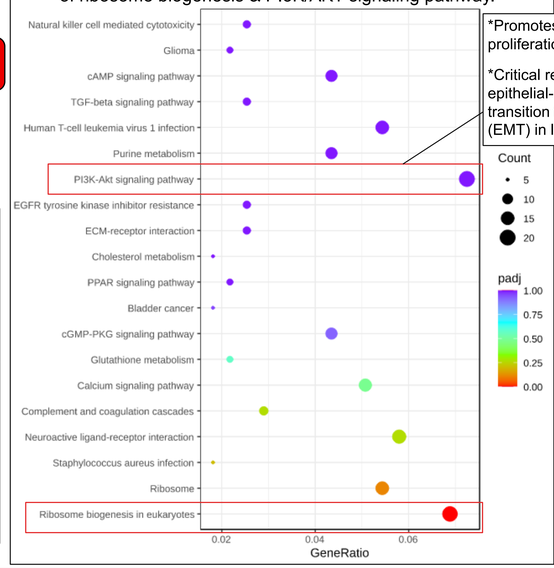
Figure 5: 1,3-BD exposure significantly upregulates mRNA expression of *GREM1* & *SERPINB2*.



GREM1 – Bone morphogenic protein (BMP) antagonist
- Linked to inflammation, fibrosis, & several cancers
- High expression → poor prognosis in cancers[9,10]
- Stimulates PI3K/AKT signaling pathway[11]
  → epithelial-mesenchymal transition (EMT)

SERPINB2 – Plasminogen activator inhibitor-2 (PAI-2)
- Inhibitor of cell proliferation & migration
- Low expression → reduced survival in lung adenocarcinomas[12] (independent prognostic factor)

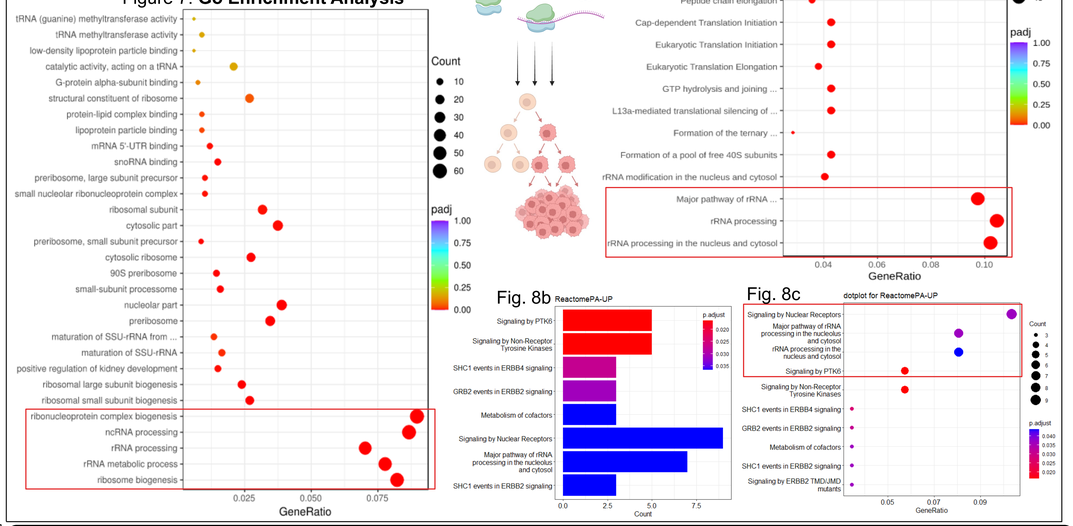
Figure 6: **KEGG Enrichment Analysis** reveals involvement of ribosome biogenesis & PI3K/AKT signaling pathway.



*Promotes tumor cell proliferation & survival

*Critical regulator of epithelial-mesenchymal transition transition (EMT) in lung cancers.

Figure 7: **Go Enrichment Analysis**



Figures 7 & 8: GO & Reactome Enrichment Analyses indicate 1,3-BD exposure is associated with early oncogenic responses

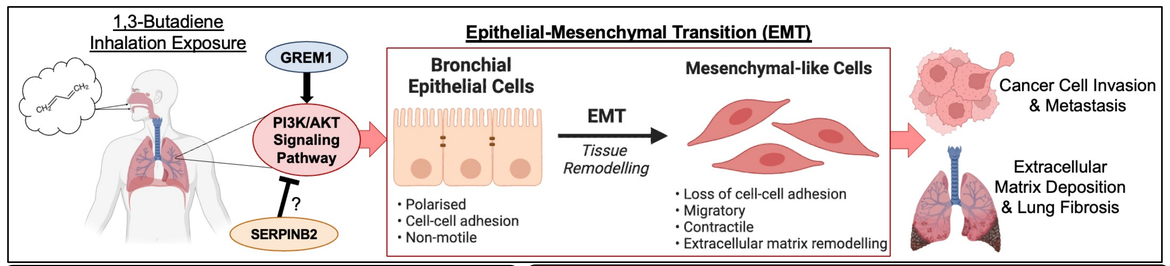
Figure 8a: **Reactome Enrichment Analysis**

Fig. 8b ReactomePA-UP

Fig. 8c dotplot for ReactomePA-UP

CONCLUSION

1,3-BD exposure may promote carcinogenesis in human bronchial epithelial cells (BEAS-2B) by activating *GREM1* to potentiate epithelial-mesenchymal transition (EMT) through stimulation of the PI3K/AKT signaling pathway. Overexpression of *SERPINB2* may serve as an initial protective mechanism to reduce cell migration & EMT.



ACKNOWLEDGEMENTS

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REFERENCES

1. Delzell et al (1996), Toxicology, 113.
2. Macaluso et al (1996), Toxicology, 113.
3. ATSDR (1992).
4. McGraw et al (2021), Environmental Research, 196.
5. Lin et al (2020), Journal of Hazardous Materials, 396.
6. U.S. EPA (2009).
7. CalEPA (1997).
8. Penn & Snyder (1993), Circulation, 88.
9. Bao et al (2023), J Cancer Res Clin Oncol, 149.
10. Li et al (2022), Cells, 11.
11. Jiang et al (2023), World J Surg Onc, 21.
12. Ramnefjell et al (2017), Oncotarget, 8.