# DIESEL PARTICULATE EXPOSURE: CROSS-TALK BETWEEN IL-17 FAMILY AND APOPTOTIC PATHWAY GENES


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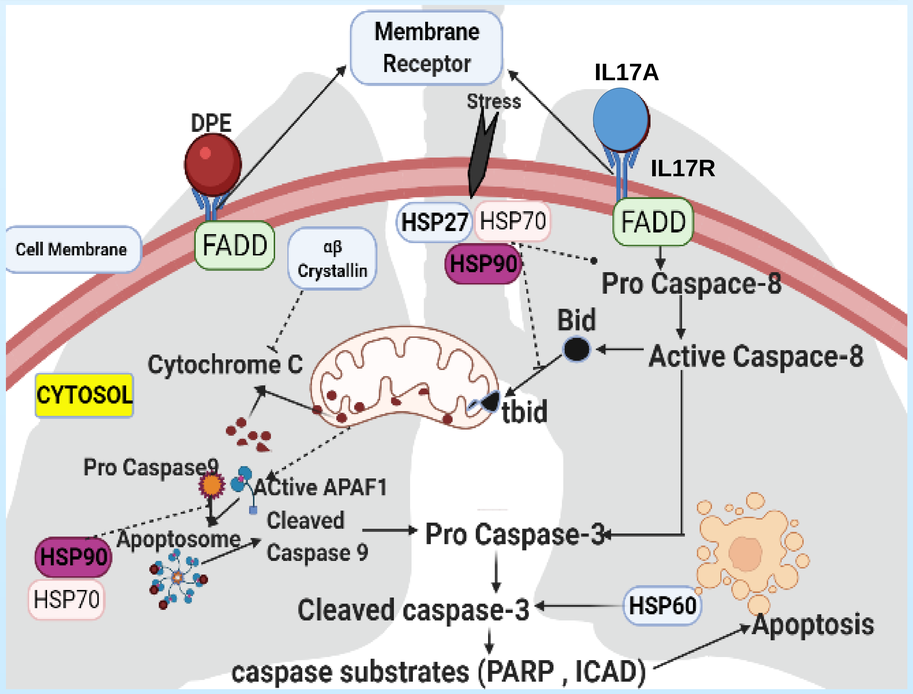
## INTRODUCTION

Pulmonary health is routinely challenged with a variety of environmental factors, that directly influence the viability and function of the lungs. One of the environmental factors is the emission from heavy diesel engine motors being used in robust mass transportation, railroads, heavy-duty combustion engines in construction, farmland, and mining industrial settings. The emission from these sources contain harmful chemicals such as benzene, chrysene, formaldehyde, nitrous oxide, and several other organic and inorganic substances. Diesel particulate matter/extract (DPM/DPE) can cause immunomodulatory effects, including inflammatory oxidative stress and apoptosis. However, the molecular mechanisms associated with DPE-induced responses are still not clear. While apoptosis (programmed cell death) is essential for normal homeostasis, maturation of the immune system, and cellular defense; earlier studies demonstrate IL-17 mediated regulation apoptosis in pathological conditions. The current study is aimed at investigating the role of IL-17 in proliferation, apoptosis, and inflammation using human alveolar epithelial cells (A549) with type II characteristics. In brief, A549 cells were treated with various concentrations of DPE (1 mM, 10 mM, and 25 mM) for 24 and 48 hours and the samples were processed to determine IL-17A, IL-17F and IL-17R transcriptional levels and the apoptotic genes. Our preliminary studies demonstrate significant increase in the transcription of IL-17A and IL-17R in DPE-challenged A549 cells. Interestingly, we also observed increased transcription of-IL-1β and TNF-α; proapoptotic genes-BAX, BAK, FADD, FAS; and executioner/effector caspase-8 and caspase-3 in DPE exposed A549 cells. Further studies are in progress to evaluate-a) DPE-induced translational changes in cytokine/chemokine levels and apoptotic markers; and b) the impact of IL-17A/IL-17F neutralization on apoptotic markers in DPE exposed A549 cells.

## HYPOTHESIS

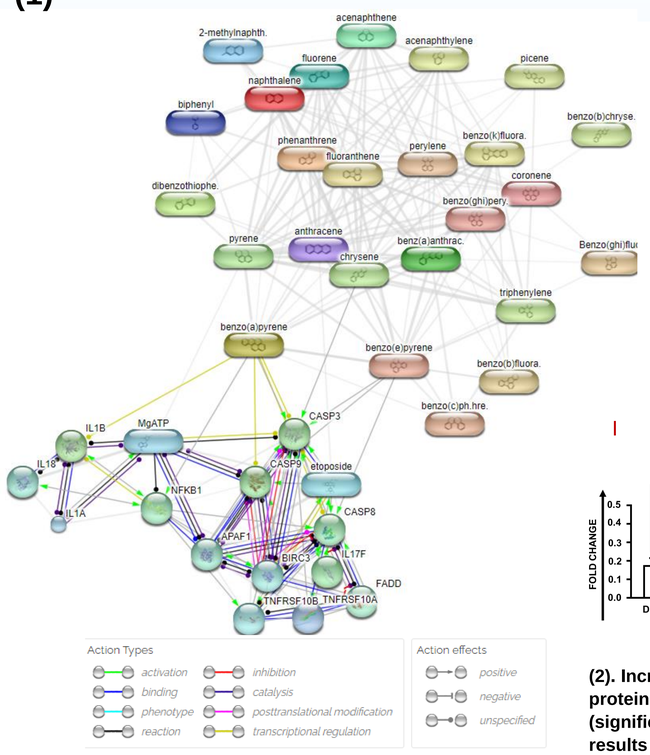
We hypothesize important role of IL17 family proteins on caspase machinery in DPE-mediated inflammation and apoptosis.

## STUDY MODEL



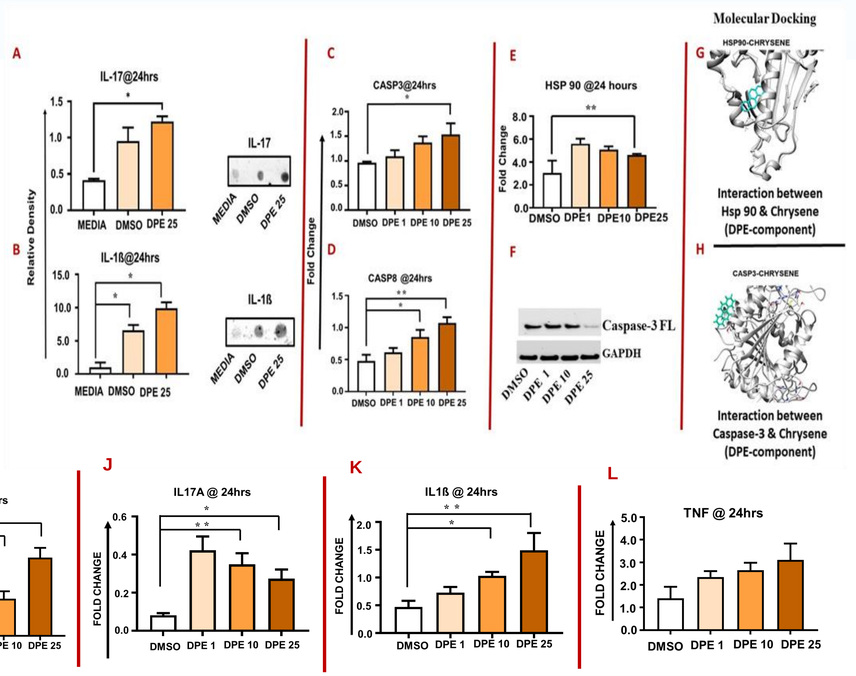

## RESULTS

(1)




Protein ligand molecular interactions network diagram of the DPE components with IL17 family and apoptotic Proteins

(2)




(2). Increased expression of: (A) cytokine IL-17; (B) cytokine IL-1β; (C) caspase-3; (D) caspase-8; and (E) heat shock protein 90 in DPE (1, 10 and 25 µg/ml) challenged A549 cells (24hr). (F) DPE-induced activation of caspase-3 (significant reduction in full length caspase-3 expression) observed at higher concentration; and (G/H) *in silico* results demonstrating the highest interaction of the DPE-component Chrysene with Hsp 90 and caspase-3.(I-L) Expression of IL17A, IL17R, IL1ß,TNFα at m-RNA level in DPE (1, 10 and 25 µg/ml) challenged A549 cells (24hr).

## METHODS

We used a hybrid in silico/invitro approach to identify the interaction of DPE components with specific apoptosis mediators/receptors and *in vitro* approach to determine the inflammatory signaling events

Computational Analysis: : The protein ligand molecular interaction was analyzed by a generating a network diagram from a cytoscape stitch web-based application and the pathways were analyzed. Further, the protein-ligand docking bond strength was analyzed by using a Auto dock suite, Swiss dock and chimera for DPE/Receptor/apoprotein interactions which were observed to be regulated by DPE.

Cell culture: Human alveolar type II epithelial (A549) cells were maintained in F-12K medium containing L-Glutamine (Corning Inc., Corning, NY) supplemented with 10% Fetal Bovine serum (FBS; Corning Inc., Corning, NY) and 1% v/v Penicillin-Streptomycin (GE Healthcare, Logan, UT) in $CO_2$ incubator at 37$^0$C .

DPE Preparation: Cells were exposed to different concentrations of DPE. Cells treated with DMSO served as control.

RNA expression analyses: cDNA was prepared from RNA isolated from treated/control cells to determine the gene expression. Relative mRNA expression was determined using 7500 Fast Real-Time PCR System (Applied Biosystems, CA) where β-Actin served as endogenous control.

Protein Expression: Enzyme Link Immuno-Sorbent Assay (ELISA) was used to determine cytokines and chemokine expressions.

## DISCUSSION

- DPE components such as Benzene, Chrysene and Nitric oxide were predicted to have higher affinity with molecular chaperones and caspase proteins, suggesting direct interactions between the DPE components and IL-17 membrane receptors.
- Furthermore, our *in-silico* results provide the basis for differential binding affinities of DPE compounds with specific receptors.
- The *in-vitro* findings from DPE challenged A549 cells corroborate with *in silico* results in terms of regulated apoptic expression.
- Specifically, we observed a concentration-dependent induction of cytokines IL-17 and IL-1ß, and NF-κβ in DPE-challenged A549 cells.
- The current findings provide critical information needed to design future studies aimed to identify the specific biomarkers or mitigation strategies for DPE induced inflammation/pathologies.

## Future Research

- Elucidate the mechanistic role of IL-17 mediated inflammation and apoptosis in DPE challenged lung epithelial cells using in silico/in vitro approach.