Paper Presentation:
Bioinformatic analysis and experimental validation of the potential gene in the airway inflammation of steroid-resistant asthma

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Background

- Steroids are the main therapy for asthma patients.
- Steroid resistant (SR) asthma develops in about 10% of asthma patients (Chung et al., 2013).
- The SR phenotype is neutrophil dependent, meaning it is immune mediated.
- Gene Expression Omnibus dataset GSE7368 contains information about SR and steroid sensitive (SS) asthmatics from Affymetrix analysis of bronchoalveolar lavage fluid (BALF).
- Prior studies indicated that pro-inflammatory, LPS signaling pathway- related genes were upregulated (EGR1, DUSP2, MAIL, TNFAIP3).

Objective

Goal 1:

Identify the top DEGs between SR and SS patients using R.

Goal 2:

Validate the role of gene DUSP2 using in vitro and in vivo models of SR asthma.

Methods – Computational

Method	Goal	Software
Differential Expression Analysis	Identify differentially expressed genes in the GSE7368 dataset	Limma R pkg (v. 3.42.2)
Tissue-Specific Gene analysis	Identify the DEGs that are tissue specific from the group of all DEGs	BioGPS (v. 94eefe6)
Functional Enrichment Analysis	Predicts the function of the DEGs' protein products	clusterProfiler R pkg (v. 3.14.3) enrichGO and enrichKEGG functions
Gene Set Enrichment Analysis	Identify sets of genes that are up/down-regulated in control vs. treatment groups	GSEA software (v. 4.0.3)
Protein-Protein Interaction Network Analysis	Identify how protein products of DEGs interact with one another	STRING (v. 10.0)
Other Statistical Analysis	Student's t-tests, ANOVA, Tukey's post hoc analysis	SPSS 19.0 software

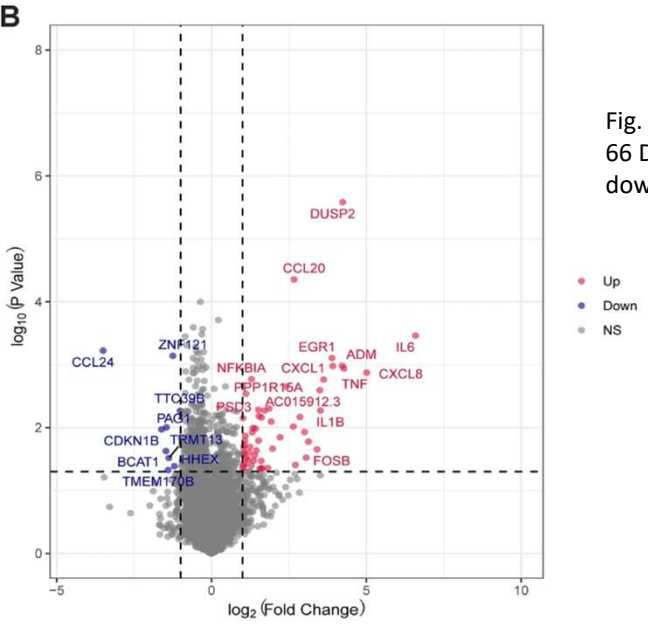


Fig. 1B: Volcano plot of genes identifies 66 DEGs. 57 upregulated and 9 downregulated.

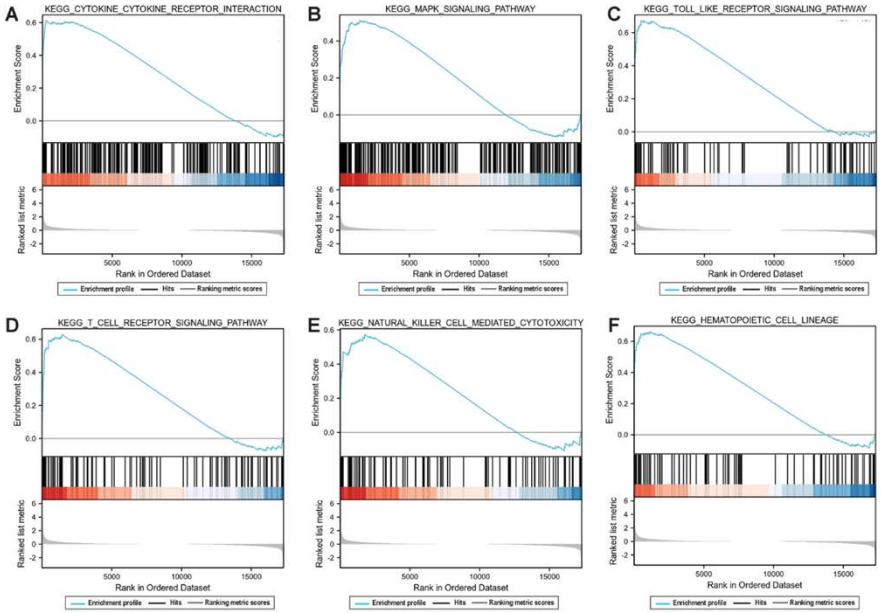


Fig. 3A-F: GSEA plots indicate the major pathways are cytokine and immune related.

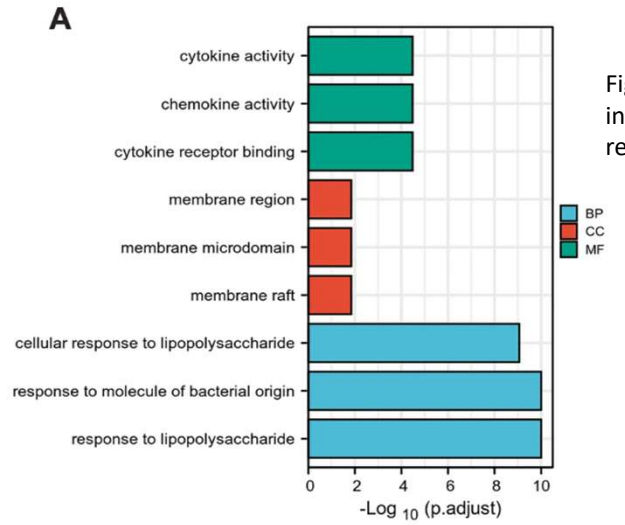


Fig. 2A: Bar plot of functional GO terms indicate significant involvement in immune response.

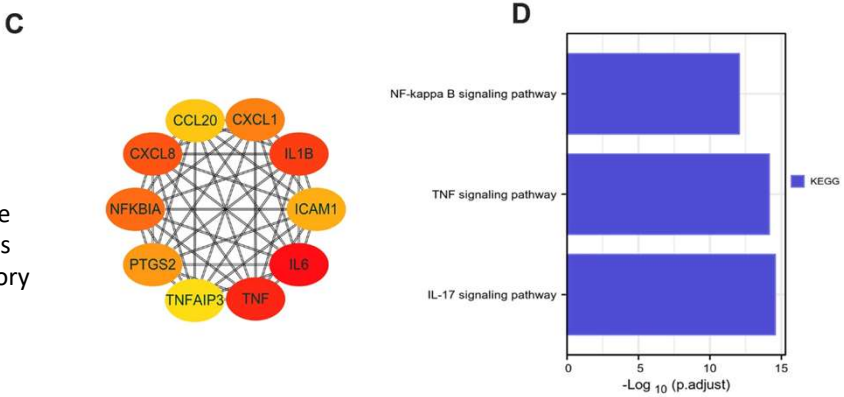


Fig. 4C, D: PPI analysis reveals the highest scoring module, C, relates to immune-mediated inflammatory pathways.

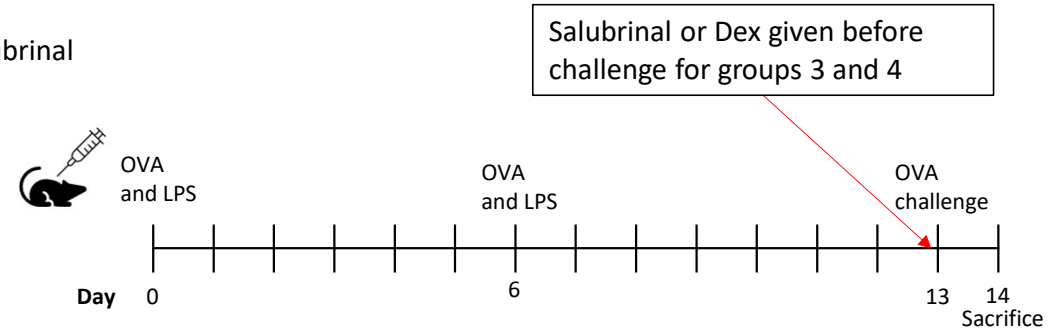
Methods – Wet Lab

Salubrinal and Dexamethsone treatment on mice

Goal: Induce steroid resistant asthma to test the effects of Salubrinal

5 groups of 4 C57BL/6 mice used

1. Control
2. Induced SR asthma
3. Induced SR asthma + Salubrinal
4. Induced SR asthma + Dexamethasone
5. Control + Salubrinal



BALF cells separated using flow cytometry, analyzed in FloJo v10

LPS and Salubrinal treatment on J744A.1 Macrophages

Goal: Induce pro-inflammatory activity using LPS to test the effects of Salubrinal

J744A.1 Macrophage Groups

Group 1 Control	**Group 2** LPS, 24 hrs
Group 3 LPS + Salubrinal, 24 hrs	**Group 4** Salubrinal, 24 hrs

Total RNA of cells extracted, and qRT-PCR used to amplify cDNA

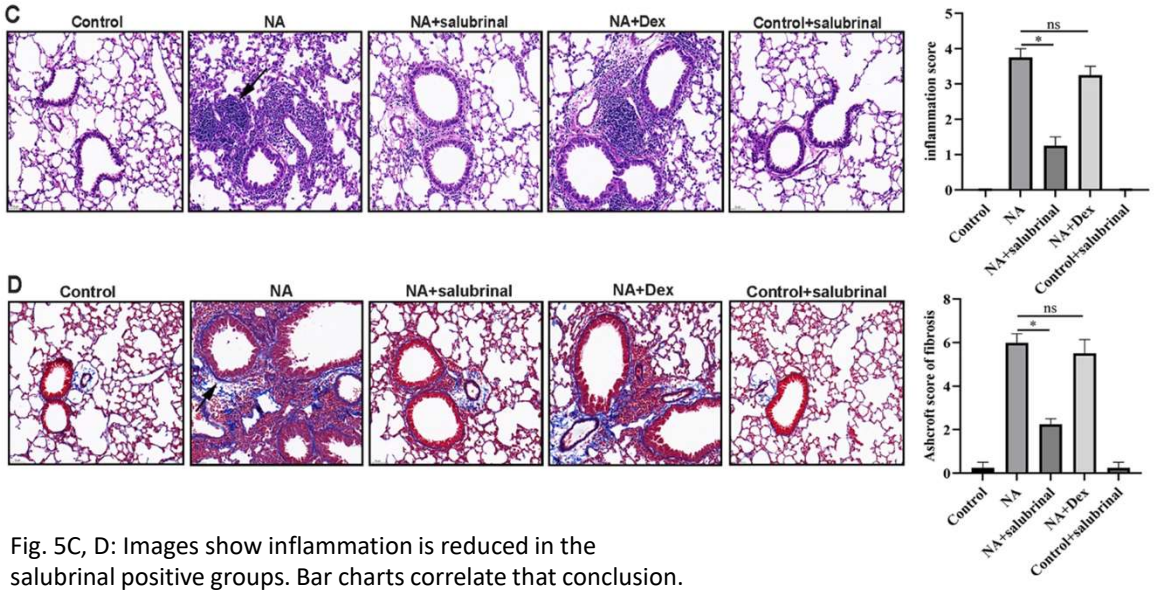


Fig. 5C, D: Images show inflammation is reduced in the salubrinal positive groups. Bar charts correlate that conclusion. Dexamethasone is unable to significantly reduce inflammation in the mouse models.

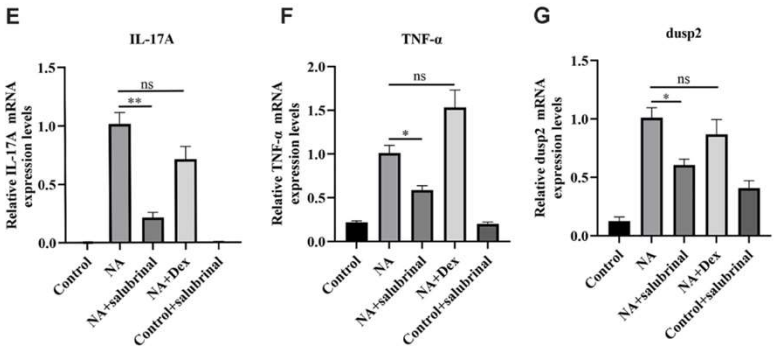


Fig. 5E-G: Bar charts of inflammatory DEG expression indicate that salubrinal significantly reduces expression of IL-17A, TNF-alpha, and dusp2.

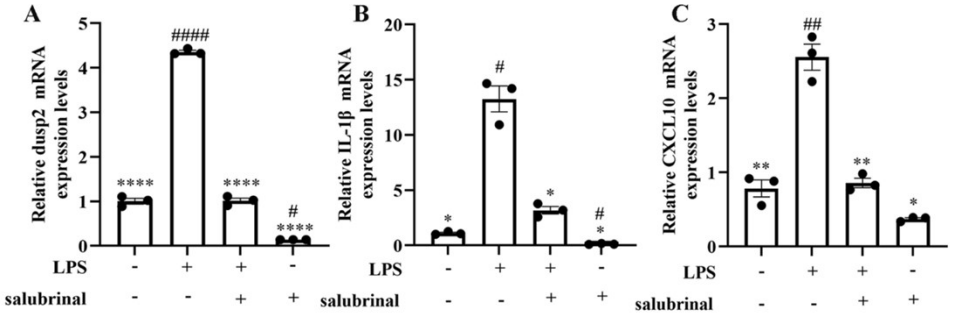


Fig. 6: Bar charts from the macrophage experiment indicate that salubrinal significantly reduces inflammatory gene expression in the presence of LPS.

Conclusions

- The study further confirms the role of neutrophils and macrophages in causing inflammation, resulting in SR asthma.
- DUSP2 expression is a key factor in SR asthma.
- Salubrinal may provide an alternative therapy for steroid-resistant asthma.

Critique

- Figure 1A seemed out of place and provided little to the paper. Figure 1B and C were far stronger for representing the DEGs between SR and SS asthma groups.

- A graphic for the tissue specific expression would have been a nice touch. All other sections for computational analysis had a figure to summarize the results. By minimizing table 3 they hide away some of their reasoning for choosing DUSP2 for testing too, as it was found to be hematologic/immune specific.

- This paper provides solid evidence that DUSP2 is involved in SR asthma pathogenesis. Future studies should work on discovering the relationship between DUSP2 and the highly scored DEG products in figure 4C.

References

- Chung, K., Wenzel, S., Brozek, J., Bush, A., Castro, M., & Sterk, P. et al. (2013). International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *European Respiratory Journal*, *43*(2), 343-373. doi: 10.1183/09031936.00202013
- Goleva E, Hauk PJ, Hall CF, Liu AH et al. Corticosteroid-resistant asthma is associated with classical antimicrobial activation of airway macrophages. *J Allergy Clin Immunol* 2008 Sep;122(3):550-9.e3. PMID: 18774390
- Wei, C., Wang, Y., & Hu, C. (2023). Bioinformatic analysis and experimental validation of the potential gene in the airway inflammation of steroid-resistant asthma. *Scientific Reports*, *13*(1). doi: 10.1038/s41598-023-35214-4