

Failed apoptotic extrusion drives an epithelia-intrinsic inflammation program.

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1. Background

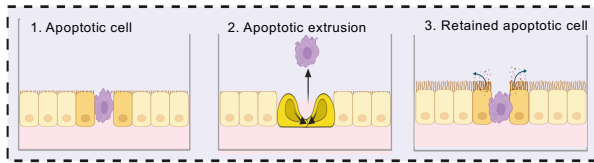


Figure 1. Schematic of epithelial apoptotic extrusion.

(1) Apoptotic extrusion a mechanically active process that removes apoptotic cells in an immunologically silent manner whilst preserving barrier integrity in epithelia.

(2) Neighbour cells generate compressive forces on the apoptotic cell. E-cadherin (CDH1) is necessary for this process, and its disruption compromises extrusion leading to retained apoptotic cells (*Duszyc et al., 2021*).

(3) This leads to upregulation of *CXCL8* expression in neighbours (*Duszyc et al., 2023*).

2. Methods

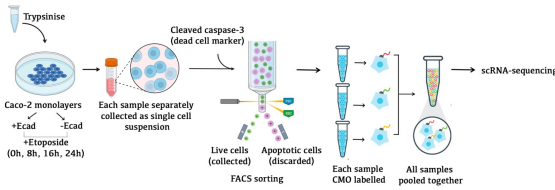


Figure 2. Schematic of the experimental pipeline. WT and siCDH1 Caco2 cells were treated with Etoposide. Apoptotic cells were discarded and samples sent for scRNA sequencing. (Dr. Zoya Mann)

3. Etoposide siCDH1 Caco2 cells upregulate inflammatory cytokines

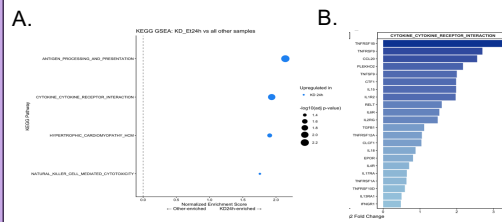


Figure 3. Inflammatory genes enriched in late KD cells. A. KEGG GSEA of KD_24hours. B. Genes contributing significantly to cytokine-cytokine receptor interactions.

4. Defining a near neighbour as 01 Inflammatory cluster

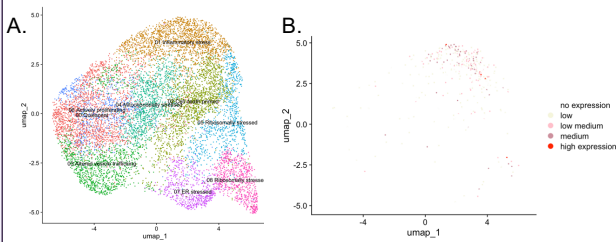


Figure 4. CXCL8 expression is enriched in 01 Inflammatory cluster. A. UMAP coloured by cluster annotations. B. UMAP coloured by CXCL8 expression

5. 01 Inflammatory cluster is enriched in Etoposide treated siCDH1 cells

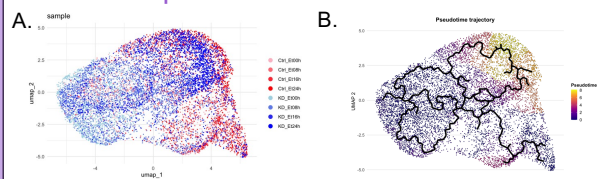


Figure 5. 01 Inflammatory cluster is enriched in Etoposide siCDH1 cells. A. Stacked bar plot showing group contribution to clusters. B. UMAP coloured by pseudotime trajectory.

6. 01 Inflammatory cluster shows an enrichment in TNF signalling pathway

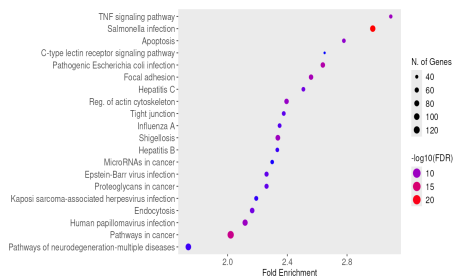


Figure 6. TNF signalling pathway is enriched in 01 Inflammatory cluster. Top 20 KEGG pathway enrichment pathways in 01 Inflammatory clusters.

7. 01 Inflammatory cluster is more similar to Crohn's disease patients than to healthy controls

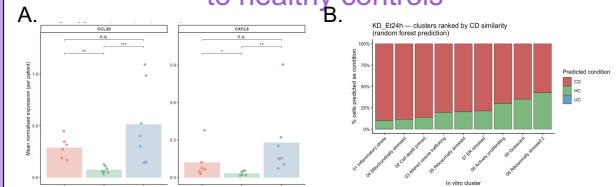


Figure 7. 01 Inflammatory cluster is transcriptionally similar to epidermal cells of Crohn's disease patients. A. CXCL8 and CCL20 expression in patient samples (*Garrido-Trigo et al., 2023*). B. Random forest machine learning algorithm showing similarity of all clusters to patients with CD, UC and HC

8. Conclusions

- ✓ The neighbours of retained apoptotic cell drive an epithelia-intrinsic inflammatory programme.
- ✓ This is driven by TNF signalling, potentially stimulating a further cytokine release.
- ✓ The inflammatory programme is reflective of epidermal cell signature in Crohn's disease patients.
- ✓ Further investigation required to reveal the extent of epithelia-intrinsic inflammation on disease pathology.

9. References

- (1) Duszyc K, von Pein JB, Ramnath D, Currin-Ross D, Verma S, Lim F, Sweet MJ, Schröder K, Yap AS. Apical extrusion prevents apoptosis from activating an acute inflammatory program in epithelia. *Dev Cell*. 2023 Nov 6;58(21):2235-2248.e6. doi: 10.1016/j.devcel.2023.08.009. Epub 2023 Aug 29. PMID: 37647898.
- (2) Garrido-Trigo A, Corraliza AM, Veny M, Dotti J et al. Macrophage and neutrophil heterogeneity at single-cell spatial resolution in human inflammatory bowel disease. *Nat Commun* 2023 Jul 26;14(1):4506. PMID: 37495570.
- (3) Duszyc K, Gomez GA, Legendijk AK, Yau MK, Nanavati BN, Gliddon BL, Hall TE, Verma S, Hogan BM, Pitson SM, Fairlie DP, Parton RG, Yap AS. Mechanotransduction activates RhoA in the neighbors of apoptotic epithelial cells to engage apical extrusion. *Curr Biol*. 2021 Mar 22;31(6):1326-1336.e5. doi: 10.1016/j.cub.2021.01.003. Epub 2021 Feb 12. PMID: 33581074.

