GLOBAL RESEARCH IMMERSION PROGRAM FOR YOUNG SCIENTISTS

CELL INJURY IN INFLAMMATORY PROCESSES

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Infection & Cell Death

Background:

During infection, ZBP1 and RIPK3 are proteins involved in necroptotic cell death that induces inflammation and restrains viral replication. Severe inflammation can lead to tissue damage and chronic diseases *[1]*.

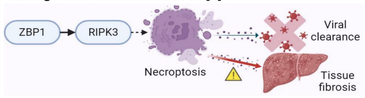


*Fig 1. Regulated necroptosis promotes viral clearance while excessive necroptosis can lead to organ damage. *RIPK3: Receptor Interacting Protein Kinase 3, ZBP1: Z-DNA-binding protein.*

Question: Which clinical pathologies involve necroptosis and how do ZBP1 and RIPK3 contribute to pathogenesis?

Methods:

Establish independent mouse reporter systems expressing fluorescent constructs of RIPK3 and ZBP1.

Fig 2. Mouse expressing fluorescent-tagged RIPK3 and ZBP1. Cells can be retrieved and viewed under confocal microscope.

Results:

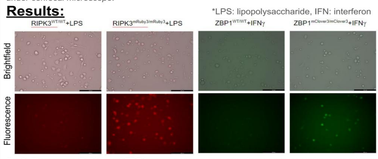
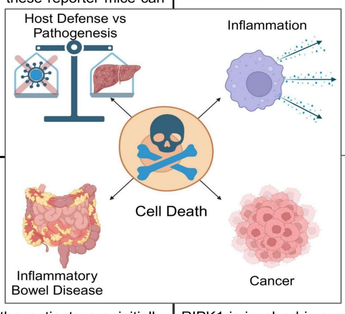


Fig 3. Fluorescent RIPK3 and ZBP1 are expressed in murine bone marrow-derived dendritic cells.

Conclusion:

LPS and IFN can stimulate fluorescent-tagged RIPK3 oligomerisation and ZBP1 expression respectively in BMDCs. In future diseased models, these reporter mice can be used to visualise how they are involved in the pathogenesis process. When activated, nuclear export of these fluorescent constructs into the cytoplasm allows them to engage downstream cell death-executing machineries.



Inflammation Regulation

Background:

Inflammation is a key immune response to infectious non-self and altered self. The activation of NF-κB induces inflammatory cytokine production. N4BP1 is a ubiquitin-binding endoribonuclease which negatively regulates the production of certain cytokines and chemokines through TLRs that signal through the adaptor protein MyD88 *[2]*. N4BP1 interacts with the NF-κB signalling essential modulator (NEMO) *[3]*. Upon cleavage of N4BP1 by caspase-8 allows the production of the inflammatory cytokines.

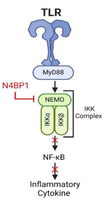


Fig 3. N4BP1 inhibits TLR-dependent activation of NF-κB

Question: In previous studies, we identified a potential target protein that interacts with N4BP1. We want to investigate whether the protein regulates the N4BP1 suppression on NF-κB pathway.

Methods:

Immunofluorescence (IF) staining was used to detect the subcellular localization of N4BP1 in different context.

Results:

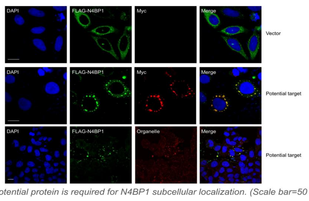


Fig 4. The potential protein is required for N4BP1 subcellular localization. (Scale bar=50 μm)

Conclusion:

The IF staining showed that the potential protein overexpression could induce N4BP1 puncta formation. The N4BP1-interacting protein may be involved in N4BP1-mediated immune regulation that can be a future therapeutic target for inflammatory diseases.

Crohn's Disease

Background:

Anti-TNF (tumour necrosis factor) therapy targeting proinflammatory cytokines TNF has been used to treat severe Crohn's disease (CD).

However, approximately 40% of the patients are initially refractory to anti-TNF therapy or lose response over time *[4]*.

Question: Since intestinal epithelial and stromal cells are cells that suffer from injury in CD, is there any marker genes expressed in these cells that can be used to differentiate anti-TNF responders and non-responders?

Methods:

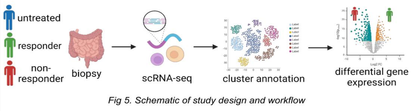


Fig 5. Schematic of study design and workflow

Results:

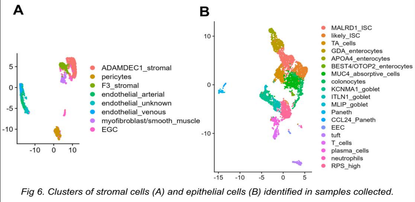


Fig 6. Clusters of stromal cells (A) and epithelial cells (B) identified in samples collected.

Conclusion:

8 clusters of stromal cells and 19 clusters of epithelial cells have been identified. By studying the differentially expressed genes between responder and non-responder in each cluster, predictive biomarkers could be discovered. Subsequent patient stratification can help optimise resource allocation and prevent delay of treatment in non-responders.

Cancer

Background:

The resistance of cell death is a hallmark of cancer, allowing cancer cells to proliferate uncontrollably *[5]*. Key mediators of cell death pathways include RIPK1 and GPX4.

RIPK1 is involved in apoptosis and necroptosis, while GPX4 regulates ferroptosis *[6]*. Aberrant regulation of RIPK1 and GPX4 can contribute to tumour development by enabling cancer cells to evade cell death.

Question: What proteins interact with RIPK1 and GPX4 in tumour development?

Methods:

TurboID-based proximity labelling

- TurboID is an engineered biotin ligase that rapidly biotinylates proximal proteins in living cells.
- High affinity between biotin and streptavidin enables isolation of biotinylated proteins.

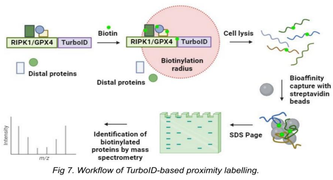


Fig 7. Workflow of TurboID-based proximity labelling.

Results:

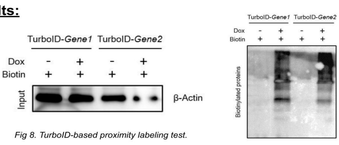


Fig 8. TurboID-based proximity labeling test.

Conclusion:

Once interaction partners of RIPK1 and GPX4 have been identified, relevant interaction networks and functional roles in cancer models can be mapped. This can be useful in identifying novel therapeutic targets for cancer treatments.

Key References

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3. Shi, H., et al., N4BP1 negatively regulates NF-kappaB by binding and inhibiting NEMO oligomerization. Nat Commun, 2021. 12(1): p. 1379.
4. Schmitt, H., et al., Expansion of IL-23 receptor bearing TNFR2+ T cells is associated with molecular resistance to anti-TNF therapy in Crohn's disease. Gut, 2019. 68(5): p. 814.
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Diagrams used in this poster were created on BioRender.com.

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