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MATRIPTASE ACTIVATION OF GQ DRIVES EPITHELIAL DISRUPTION AND INFLAMMATION VIA RSK AND DUOX

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INTRODUCTION

Fig. 1: Zebrafish epidermal mutant hai1a

- Matriptase overexpression implicated in human carcinoma and inflammation
- In zebrafish, mutation of Matriptase inhibitor HAI-1 results in epidermal defects and sterile inflammation[2]

HAI1 ⊣ MATRIPTASE1

- Matriptase activates Par2 and signals through Gq as canonical target, linking with intracellular calcium ions via phospholipase C[3]
- Pathological implications of the signalling pathways downstream of Par2 remain unclear and are of clinical interest

Aim: To map essential pathways downstream of zebrafish Matriptase and Par2 that causes inflammation and epithelial defects

METHODS

Inhibitors	Antibodies	Analogues	Stainings
2APB (IP3-R)	pERK	PMA (DAG)	PFBSF
DPI (Duox)	p90RSK		
YM-254890 (Gq)			
UO126 (pERK)			
CI-1040 (pERK)			
Dimethyl fumarate (pan-RSK)			
BI-D1870 (pan-RSK)			

Table 1: List of relevant inhibitors, antibodies, analogues and stainings used in this study

- Various reagents (Table 1) were dissolved in DMSO, diluted in embryo medium and Zebrafish HAI-1a mutant embryos were treated by immersion.
- Fish were housed at the IMCB and the NTU zebrafish facilities
- Embryos were aligned on an agarose plate and injected at the one-cell stage with RNA or morpholino diluted in Phenol Red and Danieau's buffer
- The embryos were imaged under fluorescent microscopy in the NTU EMB facility.

RESULTS

1. Increased **hydrogen peroxide** and **calcium flashes** contribute to inflammation in HAI-1a mutants
   - This was confirmed with inhibition of **Duox**
   - IP3R inhibitor reduced the number of calcium flashes

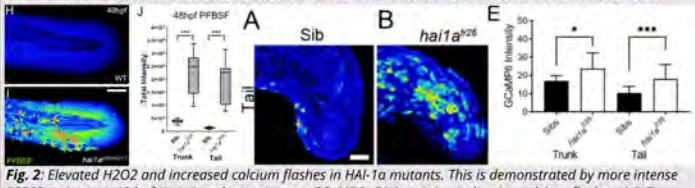

Fig. 2: Elevated H2O2 and increased calcium flashes in HAI-1a mutants. This is demonstrated by more intense PFBSF staining at 48 hpf (H, I, J) and more intense GCaMP6s RNA staining indicating calcium flashes (A,B,E)

2. Both **inflammation** and **epidermal aggregates** of HAI-1a mutants are resolved by **Gq inhibition**

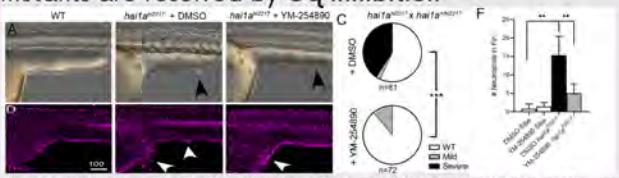

Fig. 3: Gq inhibition resolves all HAI-1a phenotypes. Epidermal defects resolved (A,B,C) and inflammation resolved (D,F)

3. PMA treatment phenocopies the HAI1a mutant, PKC inhibitor rescued
4. Elevated MAPK signalling generates epithelial defects in HAI-1a mutants
5. **Phosphorylation of cytoplasmic RSK** by pERK leads to **loss of E-cadherin** at the HAI-1a keratinocyte membrane

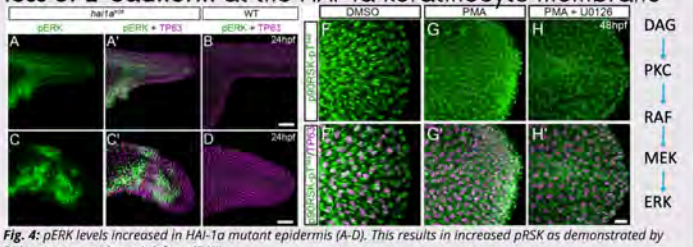

Fig. 4: pERK levels increased in HAI-1a mutant epidermis (A-D). This results in increased pRSK as demonstrated by PMA, causing epidermal defects (F-H')

DISCUSSION

- Inhibition of Gq and PLC reduces downstream signalling of Par2
- Different products of PIP2 hydrolysis invokes 2 main HAI-1a phenotypes: Reduction of IP3R-dependent Ca release attenuated inflammatory defects while PKC inhibition rescued epithelial defects
- PMA (DAG), also contributes to inflammation via Duox
- pERK signalling, through RSK members contributes to dissolution of adherens junctions & epithelial phenotype

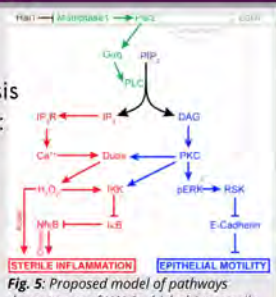

Fig. 5: Proposed model of pathways downstream of HAI-1 which drives sterile inflammation & epithelial motility

CONCLUSION

- Study has established the signalling pathway that lead to sterile inflammation and epidermal defects
- HAI-1A mutations can be used as a novel damage sensor to predict sterile inflammation and epithelial defects
- Ultimately, Gq inhibition can be deployed to reduce BOTH sterile inflammation and epithelial defects in HAI-1a mutants
- It is also of interest to find out how RSK internalises adherens junctions

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