Pseudouridine Synthases in Intestinal Stem Cell Homeostasis

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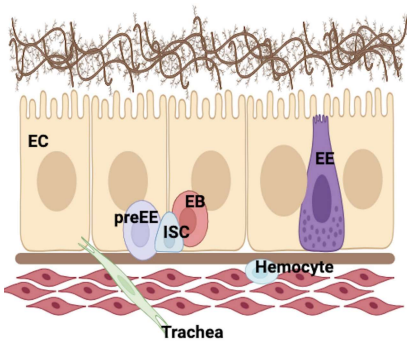
INTRODUCTION

Stem cells are unspecialised cells which can replicate indefinitely and differentiate into specialised cells. This makes them key for tissue growth and repair, particularly in the gut which has a very high rate of turnover. Excessive replication can however lead to diseases like cancer.

Pseudouridine is a modified nucleoside prevalent in many forms of RNA, affecting its structure. As non-coding genetic material impacts stem cell regulation, modifications are linked to disease prevalence, with pseudouridine synthases associated with higher incidence of certain cancers.

New model systems are needed to understandl PUS function.



This study investigated the function of Pseudouriding synthase (PUS) genes in the *Drosophila melanogaster* midgut through gene knockdown.

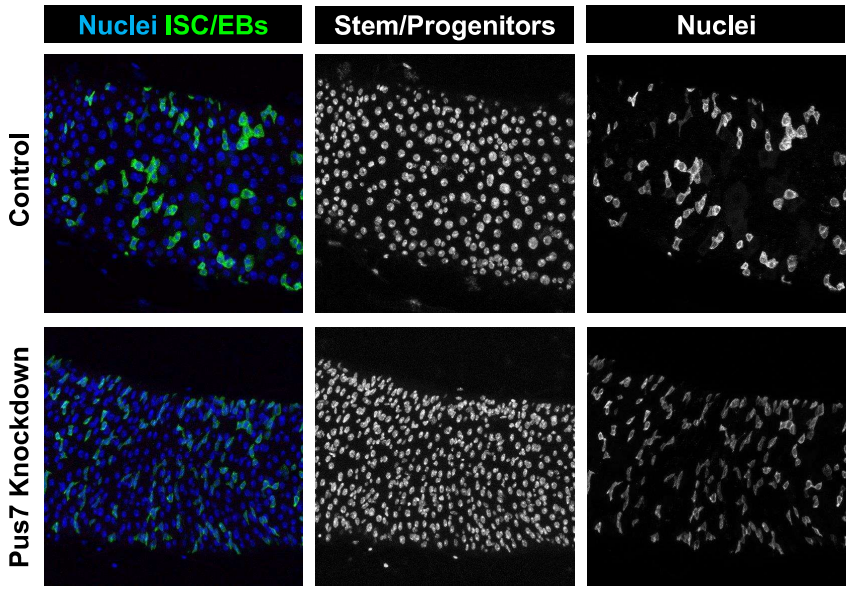
The midgut epithelium is constantly turned over throughout adult life. Differentiated cells are continuously lost to the lumen and must be replaced by the proliferation of stem cells. Intestinal stem cells self-renew and produce differentiated progeny: absorptive enterocytes and secretory enterendocrine cells.

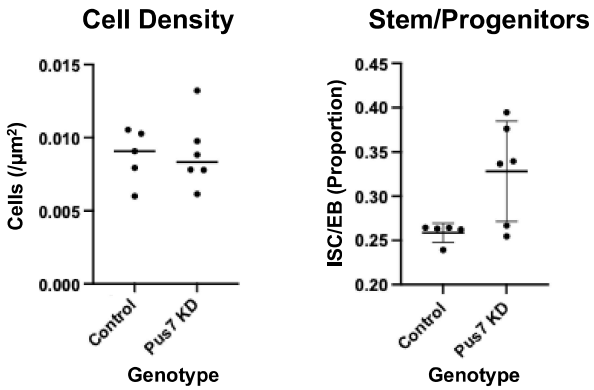
While many regulators of ISCs have been identified the function of PUS genes in midgut homeostasis has not been explored.

METHODS

- An inducible, stem/progenitor specific driver line esg-GAL4, UAS-GFP, tub-GAL80^{ts} was crossed to a PUS7 RNAi line, or a control line (W1118).
- Female progeny of these crosses were aged to adulthood at 18^{o}C, then knockdown was then induced at 29^{o}C for 7 Days
- The guts were dissected and stained with a GFP antibody (marking stem and progenitor cells) and DAPI (all nuclei).
- The guts were mounted onto slides and imaged using a confocal microscope. images produced were analysed using the software Fiji.

RESULTS





Stem / progenitor specific knockdown of pus7 results in:

- No change in overall midgut cell density.
- A significant increase in stem/progenitor cell proportion.

CONCLUSION

Pus7 is a candidate regulator of intestinal stem and progenitor cell fate. Knockdown of Pus7 results in increased stem and progenitor cell numbers which may indicate increased self-renewal or reduced differentiation.

ONGOING AND FUTURE DIRECTIONS

Characterisation of Pus7 Function: Validate knockdown result with independent RNAi lines / knockout / overexpression studies

Distinguish proliferation and differentiation effects through additional staining and lineage tracing

Identify downstream targets of Pus7 function and characterize molecular mechanisms

Identifying Novel Candidate Regulators: RNAi screen of all Drosophila pseudouridine synthases to identify those that have roles in stem and progenitor cells.

Characterise expression patterns of PUS genes to identify potential cell type specificity.

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