DEMONSTRATING WHOLE-ORGAN LINEAGE TRACING OF FLUORESCENT MARKERS IN INTESTINAL STEM CELLS USING WIDE-FIELD FLUORESCENCE IMAGING IN A ZFP148CREERT2 MOUSE MODEL

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Background

Lineage tracing using fluorescent reporters is a common tool for monitoring expression of genes and transcription factors in stem cell populations. For example, ZBP-89 (mouse Zfp148) is a transcription factor that plays a role in GI stem cell maintenance and cellular differentiation [1]. Previous studies using a Zfp148CreERT2 transgenic line demonstrated expression of ZBP-89 in both intestine and colonic stem cells and provided insight into how ZBP-89 protein expression contributes to the formation of colonic adenomas and progression during the early stages of colon cancer [2].

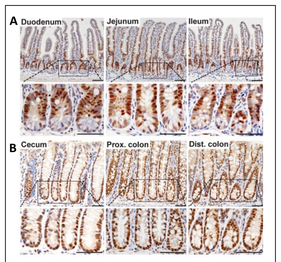

Fig 1. Representative images of IHC for ZBP-89 in the (A) small intestine and (B) colon. Expression is primarily in the crypts. Scale bar = 100 um. [2]

While lineage tracing is a useful tool, it is commonly done with high magnification microscopy, limiting the generalizability of observations given the small field of view and thin sections.

Furthermore, this requires extensive tissue processing, which is time consuming and requires sacrificing the animal. Additional knowledge could be elucidated by measuring expression of labeled markers across entire organs and with minimal tissue processing.

Objective

Our objective is to demonstrate the use of wide-field fluorescence imaging to perform rapid whole-organ, lineage tracing of ZBP-89 in a mouse model.

Methods

- We generated a cohort of tdTomato-labeled Zfp148CreErt2 mice with wild type littermates.
- Tamoxifen was injected to induce the tdTomato Cre.
- Mice were euthanized by C02 inhalation, organs were explanted.
- A wide-field fluorescent imaging system (Fig 2) was used to measure tdtTomato fluorescence in the GI tract, stomach and liver.
- Measurements taken at 24 hours, 3 weeks, 6 weeks and 12 months.

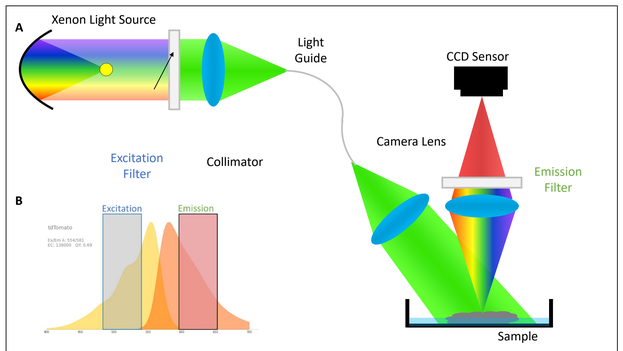

Fig 2. Diagram of wide-field fluorescence imaging system. Excitation and emission filters are tuned to the spectrum of tdTomato. Samples are imaged *ex vivo* for different portions of the GI tract including stomach, colon, duodenum, ileum, jejunum, and colon. [3]

- Images were dark subtracted and normalized by light source power and a flat-field image to correct for illumination nonuniformity.
- Regions of interest were drawn for each organ using ImageJ.
- The average grey value of the region of interest was calculated to quantify the fluorescent intensity (Fig 3).
- Tissues were cryopreserved and sectioned, and inspected under a fluorescent microscope for ground-truth comparison.

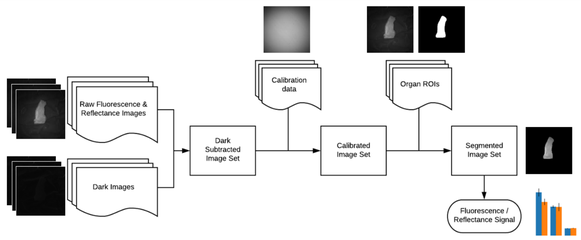

Fig 3. Quantification of images is accomplished by first calibrating the raw data, then extracting regions of interest corresponding to different organs, and calculating mean fluorescent signal.

Results

- Our results confirm that ZBP-89 is expressed ubiquitously in the gastrointestinal tract (Fig 4).
- The wide-field fluorescence results show concordance with fluorescence microscopy - we see that the level of fluorescent signal correlates with the abundance of cells expressing the labeled marker (Fig 5).

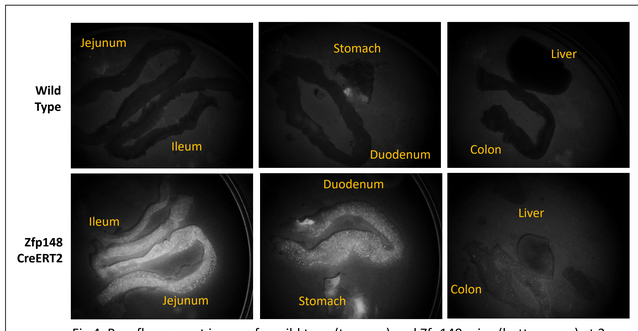

Fig 4. Raw fluorescent images for wild type (top row) and Zfp148 mice (bottom row) at 3 weeks of age. There is clear increase in fluorescent signal in the transgenic mice.

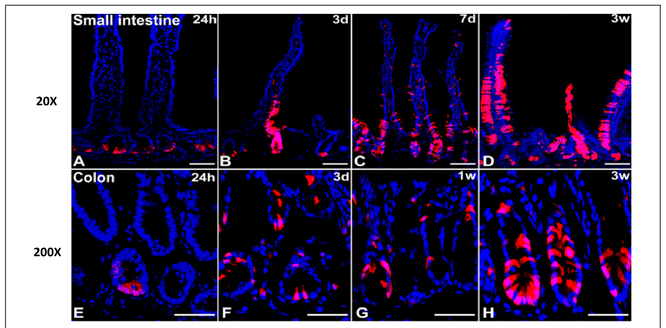

Fig 5. Cryosections of Zfp148 mice at different time points illustrating tdTomato expression in different regions of the GI tract, showing increased expression at later time points [2].

- We observe a small amount of tdTomato expression in the liver, which is consistent with literature showing Zfp148 in hepatic cells [4].
- Quantification of the images shows statistically significant increase of fluorescent signal across the GI tract between transgenic and wild type animals (Fig 7).
- We observe a gradient of decreased expression from proximal intestine to distal, suggesting increased differentiation in the proximal intestine.

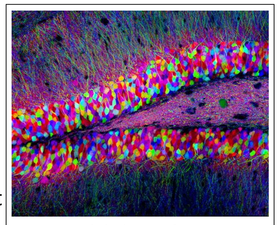

Fig 8. Example high magnification image of a tissue with multiplexed fluorescent markers, which could be used in future studies to monitor several markers [5].

Conclusions

Ultimately, we demonstrate that wide-field fluorescence imaging is a valuable tool for monitoring whole-organ expression of labeled markers. This technique could potentially be applied *in vivo* for longitudinal assessment of a single animal, further increasing the translation and impact of lineage tracing. Further innovation could be made by monitoring multiplexed fluorescent labels (Fig. 8) [5].

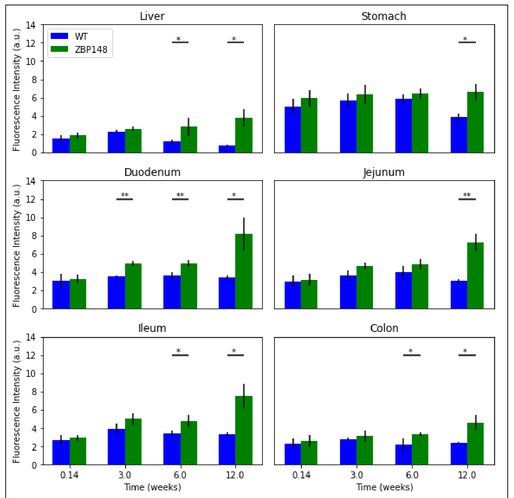

Fig 7. Quantification of wide-field fluorescence images shows a trend of increased expression at later timepoints in Zfp148 mice, which is consistent with observations made with IHC. We also observe increased expression in proximal intestine compared to distal, as well as in the liver.

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