Investigation of age-dependent changes in the enteroendocrine cells in the fruit fly *Drosophila melanogaster*（ショウジョウバエの腸内分泌細胞の加齢に伴う変化）

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Introduction

The intestine is a complex and dynamic organ responsible for nutrient absorption and digestion throughout our lifetime. Given its interaction with both external and internal environments, its homeostasis is essential for our health.

In the fruit fly *Drosophila melanogaster*, intestinal stem cells (ISCs), enteroblasts (EBs), enterocytes (ECs), and enteroendocrine cells (EECs) are the major cell types that reside in the middle part of its intestine, called midgut. Upon sensing nutritional signals, EECs can produce and secrete different combinations of peptide hormones to mediate various physiological processes linked to metabolism, immune system, and reproduction[1].

The midgut exhibits aging-induced deterioration in the maintenance of compartmentalization, associated with unrestrained ISC proliferation, impaired EC differentiation, and altered EEC hormonal expression profiles[2,3]. Although it is documented that EECs switch hormonal expression that regulates ISC activities with age[4], the linkage between an age-dependent decline in physiological functions and changes in EEC profiles is not clearly understood.

The laboratory which I belong to has been examining the role of the enteroendocrine peptide hormone Neuropeptide F (NPF) in physiological and reproductive functions[5], while much less is known about the effect of aging on NPF functioning. Using immunostaining and cell lineage analysis, this research aims to investigate how NPF-producing EECs (NPF$^+$ EECs) are altered with age in *Drosophila* and whether the change is related to age-induced loss of physiological integrity.

Materials and Methods

Aging Assay

Newly emerged virgin adult flies were collected with males and females separated under CO_2 anesthesia. 10-15 flies were placed in each vial and transferred into a fresh one every 3 days. Flies were fed on the standard diet and maintained at 25 °C.

Immunostaining and Imaging

The guts were dissected in 1× phosphate-buffered saline (PBS), fixed in 4% paraformaldehyde made in PBS for 40 min, washed three times with 0.1% PBT (PBS + 0.1% Triton-X100), blocked for 1h with blocking buffer 2% bovine serum albumin (BSA) made in 0.1% PBT, and incubated with primary antibodies, diluted in blocking buffer, overnight at 4 °C. This was followed by three washes in 0.1% PBT, incubation for 2h wrapped in foil with secondary antibodies conjugated with Alexa Fluor 488 or 546 (Thermo Fisher Scientific), diluted in blocking buffer. Guts were then washed three times for 15 min each in 0.1% PBT with 4',6-diamidino-2-phenylindole (DAPI) added before the last wash and mounted on a slide in a small drop of FluorSave (Merck Millipore). Guts were imaged on a Zeiss 900 confocal microscope (Carl Zeiss) with NPF fluorescence intensity quantified using ImageJ and the number of NPF$^+$ cells counted manually.

Cell Lineage Analysis with *Gal4* technique for real-time and clonal expression (G-TRACE)

The *Gal4/UAS* transcriptional control system is a method to monitor enhancer activities in specific cells[6]. The G-TRACE system labels current *Gal4* expression with green fluorescent protein (GFP) and descendant *Gal4* expression with red fluorescent protein (RFP)[7]. The NPF$^+$ EEC-specific *Gal4* driver line was crossed to the G-TRACE reporter stock. GFP and RFP fluorescence was examined in the guts of the crosses with immunostaining.

Results and Discussion

Immunostaining analysis showed that aging resulted in a decrease in the number of NPF$^+$ EECs and NPF fluorescence intensity in males but not in females, pointing to a sexually dimorphic age-induced change in enteroendocrine production of NPF. Furthermore, cell lineage analysis revealed that NPF$^+$ EECs could transform into ECs in aged flies, with a higher transformation ratio in females than in males.

To verify whether those age-dependent changes in NPF$^+$ EECs contribute to any age-induced decline in organismal functions, it is important to examine behavioral and physiological performances in aged flies with *NPF* knockdown and overexpression. Meanwhile, a transcriptome analysis to examine age-induced differential gene expression in NPF$^+$ EECs can be the first step to investigate the possible roles of *NPF* in aged flies at a molecular level.

References

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