Metabolic shift in gut microbiota is the potential route for cachexia prevention

Baby Bhavya Gummadi（筑波大学 生物学類）　指導教員：Yuya Sanaki（筑波大学 生存ダイナミクス研究センター）

Introduction

Cancer was traditionally studied as a genetic disease, however, increasing evidence has revealed that it has a strong impact on the host metabolism[1]. In the advanced stages of cancer, a severe metabolic disorder known as cachexia will develop, which is characterized by strong muscle wasting or/and fat mass loss. Currently, there is no established treatment for this condition, and most cases are fatal. Using *Drosophila melanogaster* as an *in vivo* cancer model, we found a remarkable gut dysbiosis in tumour-bearing flies, where the commensal *Acetobacter* and *Lactobacillus* were replaced by *Escherichia.* Strikingly, inoculating the non-pathogenic *Escherichia coli* K-12 strain was solely sufficient to induce cachexia in the tumour-bearing flies. Moreover, we found that their heat-resistant metabolites mediated the cachexia-promoting effect of gut bacteria. To identify the causative metabolites, we conducted a genetic screening targeting metabolic pathways with an *E. coli* mutant library.

Material & Methods

• Genetic Screen

Wild-type Oregon-R flies were reared on a conventional food (wheat flour, dried yeast, sugar, agar, propionic acid, methylparaben) at 25C. Unmated/virgin female flies were treated with antibiotics-containing food (Ampicillin-Gentamicin-Levofloxacin) for at least 3 days to eliminate the gut microbiota. The flies were injected with Ras^{V12} tumour cells (500 cells/fly) and then recovered in antibiotics containing food for 1 day. *E. coli* strains from the Keio collection, with a single-gene knock out[2], were orally introduced to tumour-bearing flies. And fly survival was assayed. A survival of more than 20 DAI (days after injection) was used as a criterion to consider the bacterial gene to be required for cachexia development (HIT gene).

• Histological Assay

Muscle wasting was analysed by staining flight muscles with phalloidin (Thermo Fisher Scientific) and anti-actinin primary antibody (DSHB). Fat body wasting was analysed by staining abdominal and visceral fat with Nile red (Fujifilm-Wako). Images were acquired by confocal microscope (Carl Zeiss, LSM900). The length of a sarcomere was determined by measuring the distance between z-bands in continuous muscle fibres. Fat mass loss was analysed as the density and size of lipid droplets. All image analyses were performed on FIJI.

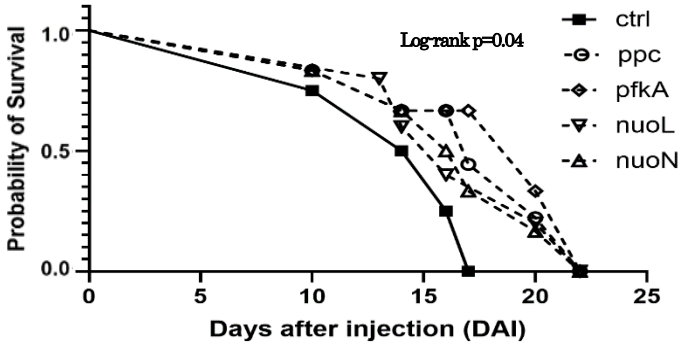
• Bacteria Characterization

The "HIT" mutants from the bacterial genetic screen were analysed for their growth in LB broth, on fly food surface, and in the host gut. Reactive oxygen species (ROS) were detected qualitatively by staining the culture with Dichloro-dihydro-fluorescein diacetate (DCFH-DA, Dojindo).

Antioxidant capability was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH, Dojindo) staining in LB broth following the manufacturer's protocol.

Results and Discussion

We found that inoculating tumour-bearing flies with *E. coli* mutant strains deficient in *nuoN*, *nuoL*, *pfkA*, and *ppc* genes resulted in prolonged host survival, suggesting that these genes are required for cachexia development.



Histological assays revealed a clear loss in fat mass upon inoculation with the control K-12 strain and the "HIT" strains. However, muscle wasting was significantly rescued in the "HIT" strains compared to those inoculated with the control K-12 strain, suggesting that muscle wasting plays a more critical role in determining survival period. The *nuo* genes are the essential components of oxidative phosphorylation, while *pfkA* and *ppc* are involved in glycolysis; both of these metabolic pathways are part of aerobic metabolism. As aerobic metabolism is the primary source of reactive oxygen species (ROS) in cells, we hypothesized that the "HIT" strains produce less ROS than the strains that induce cachexia. Furthermore, the "HIT" strains may even have antioxidant properties resulting from the activation of anaerobic pathways, allowing the host to better combat the tumour. ROS staining revealed that the "HIT" strains generate less ROS than the wild-type strain. However, this trend is not limited to "HIT" strains; certain non-"HIT" strains also exhibited low ROS levels. Additionally, the "HIT" strains demonstrated a higher antioxidant capacity compared to the non-"HIT" strains.

Hence, we propose that a metabolic shift in the gut microbiota away from aerobic metabolism contributes to the attenuation of cancer cachexia in the host, potentially through lower ROS levels and increased antioxidant production.

References

[1]Gyamfi *et al.* (2022), *Int. J Mol Sci.* 23(3):1155.

[2]Baba *et al.* (2006), *Molecular Systems Biology,* 2(1)