Title of Project： Investigation of intestinal lipid metabolism in food allergy

MURATA Takahisa
(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Associate Professor)

Research Project Number: 20H05678 Researcher Number：40422365
Keyword：Food allergy, Microbiota, Lipid mediator

【Purpose and Background of the Research】

Imbalance of intestinal microbiota (dysbiosis) due to life modernization is attracting attention as a cause of food allergy. However, it is not well understood how the dysbiosis changes host's immune response and causes allergic reactions.

In the previous studies, we reported that the bioactive lipid PGD2 is an important molecule that modulates allergic reactions by strengthening the epithelial barrier of the host and by promoting IgE production against antigens. At the same time, we found that there is a correlation between changes in microbiota and changes in intestinal lipid production profile upon food allergy. There is a possibility that lipids connect intestinal microbiota to host immunity, and changes in lipid production resulting from dysbiosis may be responsible for induction of food allergy.

In this study, we will comprehensively analyze the relationship between intestinal microbiota and host's immunity focusing on bioactive lipids, and we are revealing what disturbs this relationship and increases food allergic patients. We aim to elucidate a new immunomodulatory mechanism that will provide new insight for the treatment of food allergy.

【Research Methods】

In this study, we will clarify how intestinal microbiota influence the production and reception of bioactive lipids in mice, humans and dogs.

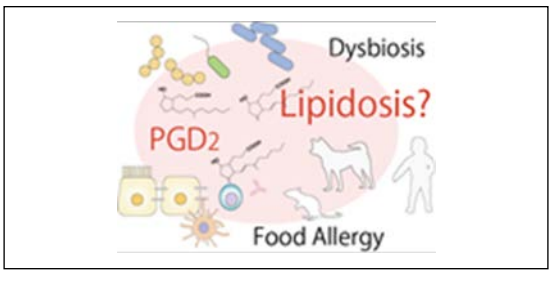

Figure 1 Analysis of the effect of dysbiosis on imunity

First, in humans, dogs, and mice suffering from food allergies, the correlation between dysbiosis and the abnormal lipid production will be clarified. We will also clarify the mechanism of how dysbiosis changes the lipid production usign murine model. Furthermore, we will explore and clarify which environmental factors and lifestyles impair the intestinal microbiota and lipid porduction. Finally, we will attempt to propose new method to improve the dysbiosis and lipid production.

【Expected Research Achievements and Scientific Significance】

The number of patients with food allergy has increased several times over the last decade. It is not known what has changed around us is increasing the disease.

In this study, we will clarify how the environment and lifestyles surrounding us change our immune responses focusing on the relationship between intestinal microbiota and lipid production, which have been attracting attention in recent years.

The results and concepts obtained in this study are not only for food allergies, but also for a wide range of diseases such as obesity and diabetes, enteritis and depression, in which dysbiosis may lead to the onset.

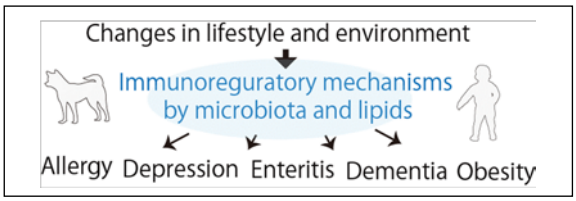

Figure 2 Scientific Significance of this study

【Publications Relevant to the Project】

・ Therapeutic potential of D prostanoid receptor 1 signal enhancement in a murine model of food allergy. #Nakamura T and #Hirai R, Tachibana Y, Masuko S, Nagata N, *Murata T. J Allergy Clin Immunol. 143(6):2290-2293. 2019.
・ 5,6-DiHETE attenuates vascular hyperpermeability by inhibiting Ca2+ elevation in endothelial cells. Hamabata T, Nakamura T, Tachibana Y, Horikami D, *Murata T. J Lipid Res. 59(10). 1864-1870. 2018.
・ PGD2 deficiency exacerbates food antigen-induced mast cell hyperplasia. Nakamura T, Maeda S, Horiguchi K, Maehara T, Aritake K, Choi B, Iwakura Y, Urade Y, *Murata T. Nature Communications. 6:7514 2015.

【Term of Project】 FY2020- 2024

【Budget Allocation】 151,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.vm.a.u-tokyo.ac.jp/houshasen/index.html

【Grant-in-Aid for Scientific Research (S)】

Broad Section F

Title of Project： **Studies on nitrogen fixation of iron reducing bacteria as a key process supporting sustainable nitrogen fertility of rice paddy soil : towards low nitrogen agriculture**

SENOO Keishi

(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Professor)

Research Project Number: 20H05679 Researcher Number：40206652

Keyword：nitrogen fixation, iron-reducing bacteria, rice paddy soil, nitrogen fertility, low-nitrogen input

【Purpose and Background of the Research】

Soil nitrogen fertility is essential for crop production. Rice paddy soil has the ability to sustain nitrogen fertility autonomously, however, its mechanism remains largely unknown. Microbial nitrogen fixation is the major route for supplying nitrogen to paddy soil, which must be a key process supporting soil nitrogen fertility. Our recent comprehensive survey showed that most nitrogen fixation genes and their transcripts in paddy soil were derived from iron-reducing bacteria. The nitrogen-fixing ability of iron-reducing bacteria isolated from paddy soils was also verified. These findings strongly suggest that iron-reducing bacteria are previously overlooked nitrogen-fixing bacteria that play a pivotal role in paddy soils.

Nitrogen fertilizers have revolutionized modern crop cultivation; however, excessive application promotes the release of nitrogen loads from agricultural fields to the natural environment. This can lead to unavoidably aggravating environmental problems including global warming and nitrate pollution in groundwater. The environmental issues caused by the nitrogen fertilizer input have provoked public concern and increased expectations for developing new methods to achieve both increased rice production and decreased environmental burden.

The principal purpose of this study is to establish the academic base of nitrogen fixation of iron-reducing bacteria in rice paddy soil. Based on the obtained information, we will propose and test novel paddy soil management strategies to increase nitrogen fixation of iron-reducing bacteria and to increase soil nitrogen fertility.

【Research Methods】

To reveal the ecology of nitrogen-fixing iron-reducing bacteria in paddy soils in detail, we will further isolate iron-reducing nitrogen-fixing bacteria from paddy soils, obtain their genomic information, and perform soil metagenomic analysis. To investigate the contribution of iron-reducing nitrogen-fixing bacteria to soil nitrogen fertility, amount of nitrogen fixation by the bacteria will be examined in both laboratory and field soils. Environmental factors regulating nitrogen fixation of iron-reducing bacteria will be analyzed; carbon compounds derived from rice plant residue decomposition and rice root exudates. Rice gene and rice plant response closely related to nitrogen-fixing activity of iron-reducing bacteria in soil will be clarified. Ferric iron compounds in soil utilized by iron-reducing bacteria as electron acceptors and generated ferrous iron compounds will be identified through several instrumental analyses. Finally, we will propose novel paddy soil management strategies to increase nitrogen fixation of iron-reducing bacteria and examine their validity by laboratory soil microcosm and field studies.

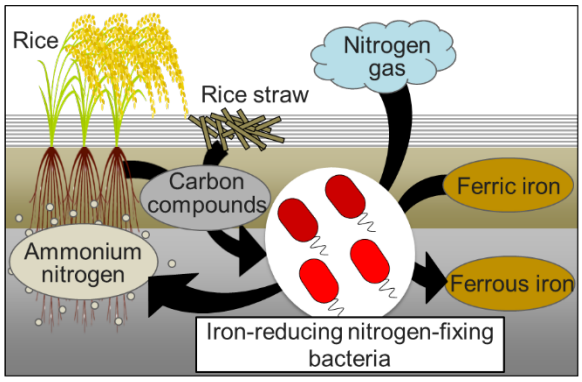

Fig. Nitrogen fixation of iron-reducing bacteria in paddy soil

【Expected Research Achievements and Scientific Significance】

The whole picture of nitrogen fixation of iron-reducing bacteria in rice paddy soil, and its contribution to sustainable soil nitrogen fertility will be figured out. This may lead to novel agricultural practices to increase soil nitrogen fertility and to ensure rice yields with reduced nitrogen fertilizer input and a lower environmental nitrogen burden.

【Publications Relevant to the Project】

・ Masuda Y, Itoh H, Shiratori Y, Isobe K, Otsuka S, Senoo K. Predominant but previously-overlooked prokaryotic drivers of reductive nitrogen transformation in paddy soils, revealed by metatranscriptomics. Microbes Environ., 32, 180-183 (2017)

・ Masuda Y, Yamanaka H, Xu Z-X, Shiratori Y, Aono T, Amachi S, Senoo K, Itoh H. Diazotrophic Anaeromyxobacter isolates from soils. Appl. Environ. Microbiol., 86, e00956-20 (2020)

【Term of Project】 FY2020- 2024

【Budget Allocation】 152,400 Thousand Yen

【Homepage Address and Other Contact Information】

http://park.itc.u-tokyo.ac.jp/soil-cosmology/

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【Grant-in-Aid for Scientific Research (S)】

Broad Section F

Title of Project： **Establishment of the basis for plant mitochondrial genome breeding**

TSUTSUMI Nobuhiro

(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Professor)

Research Project Number: 20H05680 Researcher Number：00202185

Keyword：Plant mitochondria, Genome editing, Mitochondria breeding

【Purpose and Background of the Research】

Mitochondria have their own genome of which transformation was impossible and still difficult. We recently achieved the first example of the target gene disruption of plant mitochondrial genomes using artificial nuclease TALEN with mitochondria targeting signals (mitoTALENs, Figure 1). Plant mitochondrial genomes encode genes for energy-producing oxidative phosphorylation complexes, for their expression/translation, and agriculturally highly-used cytoplasmic male sterility (CMS). Therefore, mutations and modifications of plant mitochondrial genomes should have high breeding potential. This research project has three objects for establishing mitochondrial genome breeding, 1) basic research, molecular genetics of plant mitochondrial genomes using mitoTALENs, 2) improvements and assessments of mitoTALENs, and 3) molecular studies of different systems of cytoplasmic male sterility of three plants. We try to make the base for the future breeding of unassessed plant mitochondrial genomes during the project.

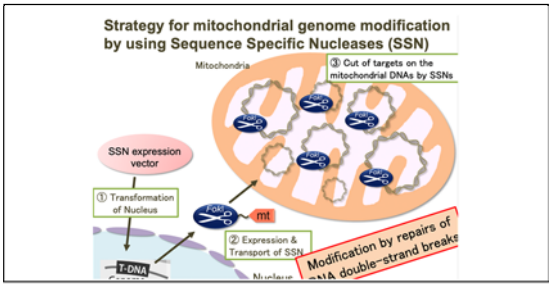

Figure 1 mitoTALEN

【Research Methods】

Mitochondrial genomes modified by mitoTALENs are good specifically-labeled genomes to trace them inside the cells, mitochondria, individuals during development, and to progenies as genetics. We use such special plants with mitochondrial-specifically tagged plants to dissect unknown transmission, replication, and maintenances of plant mitochondrial genomes.

mitoTALENs caused the targeted gene disruption with a large deletion including the target and novel connections of the sequence, resulting in new genomic structures. Such disturbance of the genomic structure might be suitable for novel mutagenesis of the genomes bringing new chimeric genes. They would be useful for a new breeding system. We will assess the phenotypes of many mutants by mitoTALENs for seeing the potential for the important agricultural traits. Improvements of mitoTALENs and their effects on the mitochondrial genomes will also be analyzed. In addition to the model plants, agriculturally important materials with CMS will be analyzed to identify the responsible genes and dissect the molecular mechanisms.

【Expected Research Achievements and Scientific Significance】

Identifying genes for CMSs in three plants and unveiling the CMS's molecular mechanisms in each plant would have significant impacts on basic research and agricultural breeding. Assessments of mutant panels of plant mitochondrial genomes would be the first step essential information for the future of mitochondrial breeding.

Plant mitochondrial genomes are multi-copy in each cell, and heterogeneous state of them in each cell was still hard to be analyzed. Our new approach dissecting basic mechanisms for maintaining the mitochondrial genome in each mitochondrion, cell, organ, and individuals to progenies will give us further insight into basic science and applied breeding systems.

【Publications Relevant to the Project】

- **Kazama T**, Okuno M, Watari Y, Yanase S, Koizuka C, Tsuruta Y, Sugaya H, Toyoda A, Itoh T, **Tsutsumi N**, Toriyama K, Koizuka N and **Arimura S** (2019) Curing cytoplasmic male sterility via TALEN-mediated mitochondrial genome editing. ***Nature Plants***, 5: 722-730.
- Method for editing plant mitochondrial genome. Arimura S, Kazama T, Katayama K, Hidaka T, Toriyama K, Tsutsumi N. JP patent-pending and US patent.

【Term of Project】 FY2020-2024

【Budget Allocation】 152,600 Thousand Yen

【Homepage Address and Other Contact Information】

http://park.itc.u-tokyo.ac.jp/pmg/

【Grant-in-Aid for Scientific Research (S)】

Broad Section F

Title of Project： Rice NLR genes, their function and evolution

TERAUCHI Ryohei
(Kyoto University, Graduate School of Agriculture, Professor)

Research Project Number: 20H05681 Researcher Number：50236981
Keyword：Rice, Blast, Resistance, Genome, Evolution

【Purpose and Background of the Research】

Rice crop feeds more than 50% of the world population. Blast disease caused by a fungus *Magnaporthe oryzae* is the most devastating disease of rice. To control blast disease, deploying rice resistance genes is the most effective mean. Plant pathogens manipulate host by secreting and injecting effector molecules to host cells. A subset of pathogen effectors are recognized by plant resistance proteins (R-proteins) coded by resistance gene (*R*-genes) and trigger strong host resistance. Such effectors recognized by the hosts are named avirulence (AVR) effectors. The majority of R-proteins are Nucleotide-binding Leucine-rich repeat Receptors (NLRs). We have isolated three *Magnaporthe oryzae AVR* genes (*AVR-Pia*, A*VR-Pii* and A*VR-Pik*) and are studying their interactions with cognate rice *R*-genes, *Pia*, *Pii* and *Pik*, respectively. Each of the three *R*-genes comprises a pair of NLR genes coding for "Sensor NLR" and "Helper NLR": *Pik=Pik-1+Pik-2*; *Pia=RGA5+RGA4*; *Pii=Pii-2+Pii-1*). Sensor NLRs contain extra integrated domains (IDs) in addition to the canonical CC, NB and LRR domains, and these IDs have similarities to domains of host proteins (Fig.1).

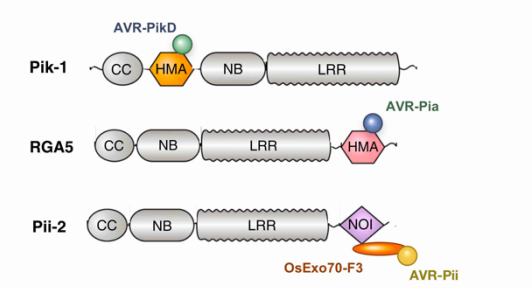

Fig.1 Recognition of *Magnaporthe oryzae* AVRs by rice "Sensor NLRs" is mediated by Integrated Domains (IDs). AVR-PikD and AVR-Pia bind HMA IDs of Pik-1 and RGA5, whereas AVR-Pii binds host OsExo70-F3 protein which in turn binds NOI ID of Pii-2.

The host proteins with ID-like domains are hypothesized to be the targets of pathogen effectors. These domains were most likely incorporated to NLR as IDs during evolution to detect pathogen effectors. Based on these findings, we set out to further understand the molecular function of rice NLRs and to engineer durable resistance against blast disease.

【Research Methods】

(1) Engineering of rice NLRs to broaden the recognition specificities: Rice *Pik-1* locus has multiple alleles with different HMA domain sequences. They show recognition specificities to variable *AVR-Pik* alleles due to differential binding of AVR-Pik variants to Pik-1 HMA variants. However, all AVR-Pik variants strongly bind to their host target proteins; small HMA proteins (sHMAs). In this project, we engineer broad spectrum Pik-1 by replacing Pik-1 HMA ID with HMA domain from sHMA. We also found that *Pia/Pias* locus has highly divergent alleles with different IDs. For instance, Pia Sensor NLR RGA5 has HMA domain, whereas Pias Sensor NLR Pias-1 has DUF761 domain in an identical position. We engineer ID of *Pia/Pias* locus by inserting domains of host proteins that are common target of pathogen effectors. Such NLR engineering is expected to confer broad spectrum resistance to rice.

(2) Functional analysis of paired NLRs: In Pia, Helper NLR (RGA4) is known to trigger hyper-sensitive (HR)-like cell death whereas Sensor NLR (RGA4) suppresses RGA4 as well as senses AVR. In Pik and Pii, Helper NLR and Sensor NLR do not seem to be involved in negative regulation and they cooperate to transduce resistance signal. In this project, we elucidate molecular mechanisms of Pia negative regulation and Pii/Pik cooperation. Additionally, we employ systematic knockout of NLRs to understand NLR regulation networks.

(3) Understanding molecular function of AVR-host target interactions: We have isolated AVRs and many effectors from *M. oryzae*. We will study their host interactors and understand molecular functions of these interactions.

【Expected Research Achievements and Scientific Significance】

Better understanding of rice NLR molecular function will allow us to engineer NLRs that may confer rice with broad spectrum resistance against blast and other diseases.

【Publications Relevant to the Project】

· Structural basis of pathogen recognition by an integrated HMA domain in a plant NLR immune receptor. Maqbool A., Saitoh H. et al. (2015) ***eLife*** doi:10.7554/eLife. 08709.002.

【Term of Project】 FY2020-2024

【Budget Allocation】 118,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.crop-evolution.kais.kyoto-u.ac.jp
http://www.ibrc.or.jp

【Grant-in-Aid for Scientific Research (S)】

Broad Section F

Title of Project： **Genome immunity: elucidation of the antiviral activity of endogenous bornaviruses and their utilization as functional resources**

TOMONAGA Keizo

(Kyoto University, Institute for Frontier Life and Medical Sciences, Professor)

Research Project Number: 20H05682 Researcher Number：10301920

Keyword：Endogenous viruses, Bornavirus, RNA, Antiviral activity

【Purpose and Background of the Research】

The genomes of our organisms contain many genetic sequences derived from viruses. In 2010, we discovered endogenous elements derived from ancient bornavirus infection (endogenous bornaviruses) in the genomes of many mammals, including humans, and suggested that our genomes have more viral-derived sequences than previously thought. On the other hand, we also demonstrated in previous studies that transcripts derived from endogenous bornavirus elements suppress exogenous bornaviral infection as RNAs or proteins. These observations suggest that our genomes possess the inherited systems of antiviral (genomic immunity) similar to the CRISPR/Cas system.

The aim of this study is to investigate in detail the molecular mechanisms of the antiviral activity of endogenous bornaviruses, and to elucidate the biological principle of genomic immunity. We also aim to create scientific and technological innovations by applying the findings from this study (Fig. 1).

【Research Methods】

We will conduct the following analyses.

(1) Elucidation of the mechanism of the antiviral activity of endogenous bornaviruses: We will perform an in-depth analysis to understand the expression profiles of endogenous bornaviruses and elucidate the mechanisms of the antiviral activity of their transcripts.

(2) Elucidation of the sequence characteristics and expression mechanism of endogenous bornavirus RNAs: To elucidate the features of endogenous bornavirus RNAs important for antiviral activity, we will investigate their structure, modification and expression in detail.

(3) Regulation of antiviral activity by modifying the RNA sequences: To manipulate the antiviral function, we will modify the RNAs produced from endogenous bornaviruses and regulate their activities in vitro.

(4) Sequence design for the regulation of antiviral activity: We will generate cell lines with various mutations in the sequences of endogenous bornavirus elements and investigate their antiviral defense characteristics.

(5) Establishment of cell lines expressing antiviral RNAs to specific viruses: To establish cell lines resistant to specific viruses, cassette constructs expressing synthesized antiviral RNAs are transduced into the cells.

【Expected Research Achievements and Scientific Significance】

This study will elucidate the biological principle of "genomic immunity" by endogenous viruses and lead to the discovery of CRISPR-Cas-like mechanism of mammals. Our research achievements will also develop antiviral drugs and vaccines based on a new principle of action, as well as establish safe transplanted cells and vaccine production cells to prevent viral contamination.

【Publications Relevant to the Project】

- Horie M, Honda T, Suzuki Y, Kobayashi Y, Daito T, Oshida T, Ikuta K, Jern P, Gojobori T, Coffin JM and **Tomonaga K**. Endogenous non-retroviral RNA virus elements in mammalian genomes. Nature 463:84-87 (2010)
- Parrish NF, Fujino K, Shiromoto Y, Iwasaki YW, Ha H, Xing J, Makino A, Kuramochi-Miyagawa S, Nakano T, Siomi H, Honda T and **Tomonaga K**. piRNA derived from ancient viral processed pseudogenes as transgenerational sequence-specific immune memory in mammals. RNA 21:1691-1703 (2015)

【Term of Project】 FY2020- 2024

【Budget Allocation】 147,200 Thousand Yen

【Homepage Address and Other Contact Information】

https://t.rnavirus.virus.kyoto-u.ac.jp

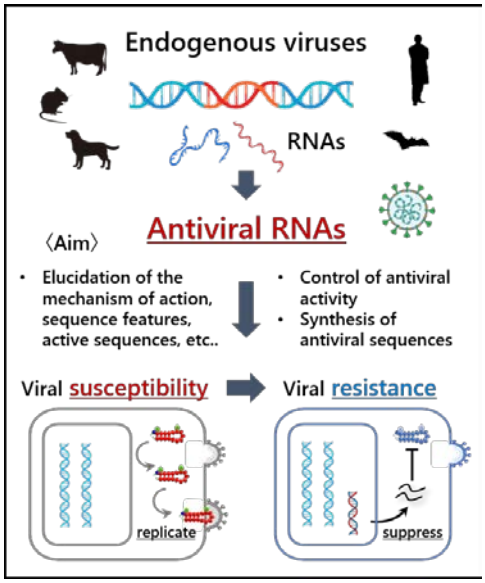

Fig.1 Genome immunity and its application

【Grant-in-Aid for Scientific Research (S)】

Broad Section F

Title of Project： Integrated understanding of food functional responsible factors and their functional interactions

TACHIBANA Hirofumi

(Kyushu University, Graduate School of Bioresource and Bioenvironmental Sciences, Professor)

Research Project Number: 20H05683 Researcher Number：70236545

Keyword：food functional responsible factors, miRNA, food factor sensing, epigenome, functional food pairing

【Purpose and Background of the Research】

Accurate understanding of "food intake", which is the most basic life activity in the maintenance and development of living organisms, is important both academically and socially. Applicants have aimed to elucidate the mechanism of bioregulatory action of food factors by considering food factors as bioregulatory signal factors and clarifying their sensing mechanism.We demonstrated that cell surface 67 kDa laminin receptor confers EGCG responsiveness to various type of cells at physiological concentrations (direct action pathway) (Fig. 1). On the other hand, the mechanism of functional expression of food factors, which are poorly absorbed and difficult to act directly on peripheral tissues and cells, is still largely unknown. In order to understand these, not only the molecules contained in food, but also the metabolites produced through living organisms and microorganisms, and the food-derived molecules that act on living organisms are regarded as " food functional responsible factors ". It is necessary to comprehensively understand the interrelationship (functional food pairing) (Fig. 2).

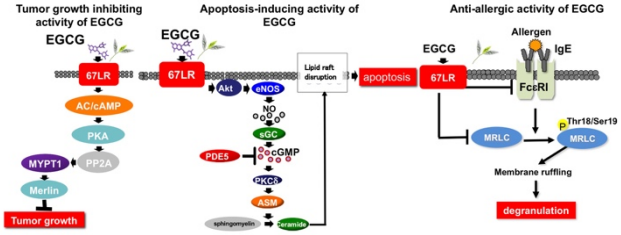

Figure 1 67LR is a critical sensor molecule to respond to EGCG and mediates the biological activities

【Research Methods】

We use liquid biopsy that captures changes in exosomes, functional RNAs, metabolites, DNA methylation, etc. in human blood. By analyzing the biological response that occurs before and after ingestion of food, we will elucidate the molecular mechanism that leads to the biological response from the food functional responsible factors. We will understand the function of food by analyzing the functional interaction between food factors. By conducting the following research items, we will elucidate the whole picture of food functional responsible factors.

1) Micro RNAs as food functional responsible factors
2) Circular RNAs as food functional responsible factors
3) Dietary plant-derived miRNA as food factors
4) Metabolites as food functional responsible factors
5) Epigenomic regulation by food factors
6) Identification of sensory molecules for hard-absorbing polyphenols
7) Elucidating of functional interactions between food functional responsible factors

【Expected Research Achievements and Scientific Significance】

It is positioned as a frontier study of "Precision Functional Food Science" that elucidates the actual state of dietary function-executive molecules and their mechanism of action, and presents scientific evidence on food intake that should be practiced for maintaining and improving health.

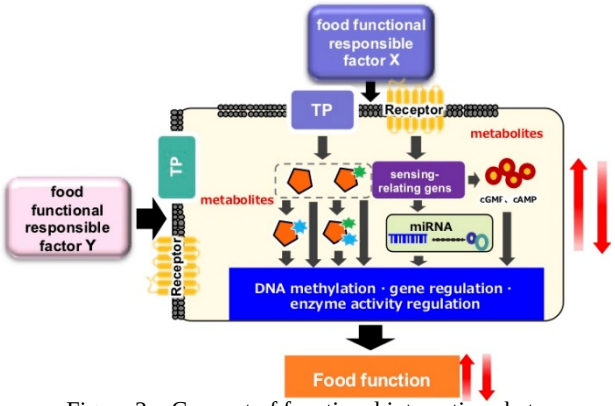

Figure 2 Concept of functional interactions between food functional responsible factors

【Publications Relevant to the Project】

・ Kumazoe, M., *et al*. 67-kDa laminin receptor increases cGMP to induce cancer-selective apoptosis. *J. Clin. Invest*., 123, 787-799 (2013)
・ Yamada, S., *et al.*, Epigallocatechin-3-*O*-gallate up-regulates microRNA-let-7b expression by activating 67-kDa laminin receptor signaling in melanoma cells. *Sci. Rep*., 6, 19225 (2016)
・ Bae, J., *et al.*, Procyanidin C1 inhibits melanoma cell growth by activating 67-kDa laminin receptor signaling. *Mol. Nutr. Food Res*., 64, 1900986 (2020)

【Term of Project】 FY2020- 2024

【Budget Allocation】 148,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.agr.kyushu-u.ac.jp/lab/syokuryo/